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Abstract Book

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Abstracts

Oral presentations

Abstract: O_1

Estimating HIV epidemic characteristics from a dated transmission network

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Background: Transmission networks constructed from densely sampled genetic data have been used to characterize the HIV epidemic. To date, temporal trends in HIV epidemic characteristics have been inferred by correlating transmission cluster composition with imputed infection times. Such imputation studies use assays of immune system kinetics which are not as ubiquitous as sequencing data. We present a method for constructing a dated transmission network which uses divergence times for estimating time of transmission. We then study two aspects of the HIV epidemic. Temporal trends in the extent of compartmentalization by transmission group and factors associated with transmission of drug resistance (TDR).

Materials and Methods: HIV pol gene sequences from 27124 patients in the Euresist database were included in this study. Sequences were subtyped using COMET and aligned using MAFFT. All major transmission routes are well represented, including 4831 MSM, 3298 IDU and 3235 heterosexual patients. Sequences from 6717 therapy naive patients were available for TDR analysis. 90% Bootstrap support and 0.02 LogDet distance were used to identify transmission clusters. Clusters were pooled in groups and for each pool, *BEAST* was used for constructing time scaled phylogenetic trees. We used the SRD06 substitution model, a log normal molecular clock and a constant growth model. Transmission networks were constructed over a range of divergence time thresholds. ML phylogenetic trees were constructed using *FastTree* and bootstrap analysis using 1000 trees was done using the python package *Dendropy*. Network analysis was done using the R packages *igraph* and *ape*.

Results:4227 (16%) patients are linked in a transmission network composed of 1509 clusters. The cluster size distribution is right-tailed with 1064 clusters of size 2, 422 between 3 and 9 and 23 clusters of size 10 and greater. Clusters durations average at 3.1 years (IQR: 1.47-4.49), with the most persistent cluster lasting 10.7 years (1999 to 2010). 30%, 77%, and 77% of transmissions involving heterosexual, IDU and MSM are within-group transmissions. Transmission edges involving IDU show two peak (2002 and 2005) while those involving MSM show a single peak at 2005. Incidence rates in cross group transmission involving Heterosexual and IDU is highly correlated with within-group transmission incidence rates for IDU. 559/6717 therapy naive patients are linked in the transmission network. TDR patients are mostly MSM (50%) and HET (15%), IDU (<1%) rest were unknown (35%). Among the DRMs present in therapy naive patients on the network, 50% conferred resistance against NRTI, 19% against PI, 12% against, NNRTI and rest (8%). All 7 TDR clusters of size 3 or more (largest 14) contained DRMs against the NRTI drug class.

Conclusions: HIV transmission clusters are short-lived, lasting 3 years on average. TDR is associated mostly with the MSM and only DRMs conferring resistance to NRTI are present in large transmission clusters. Lack of TDR in the IDU can be explained by the possible independence of the IDU and MSM epidemics. The epidemic in the heterosexuals seems to be sustained separately by the IDU and the MSM.

No conflict of interest

Abstract: O_2

HIV-1 transmission chains in a court case: use of clonal sequences to test direction and timing of transmission

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Background: Reconstruction of HIV-1 transmission chains is widely used in court cases of HIV-1 transmission and also important to understand determinants of HIV-1 transmission, with or without TDR. Reporting such cases is important in order to define methodologies and establish its reliability.

Methods: Clonal sequences from three patients (one man, his wife and his ex-wife) involved in a potential HIV-1 transmission chain were generated. Sequenced genomic regions included *gag* (790-1205 bp relative to HXB2) and *env* (7000-7356 bp HXB2). Sequences from these patients were blasted against Portuguese and publicly available databases. The most closely related sequences were included as background controls in the alignments to study the transmission chain. Population genetics analysis of such alignments was conducted using the software BEAST v1.7.4 under an optimized evolutionary model, a Bayesian skyline plot population growth model and a strict molecular clock. Bayesian phylogenetic trees were analyzed in order to understand the history of transmission between the three individuals involved.
A priori information: Patient 1 (female) and patient 2 (male) were IVDUs and married in 1995. Patient 1 was diagnosed HIV positive in 1995. Patient 2 was diagnosed in 2004. Patient 3 (female) is married to patient 2, was diagnosed in 2004 and it had been established that she was infected by patient 2. Patient 1 sued patient 2 for infecting her.

Results: Phylogenetic analysis results of the two analysed genomic regions (*gag* and *env*) were consistent. For both analyzed genes, clonal sequences of patient 2 were paraphyletic to clonal sequences of patient 3, consistent with prior knowledge that patient 2 infected patient 3. Patient 1 however, which accused patient 2 of infecting her, clustered separately from patients 2 and 3 with control sequences separating both clusters, indicating that there was a separate transmission source for this patient and discarding the hypothesis of transmission. The time of infection of each patient was estimated based on the 95% confidence interval of the tMRCA estimates of the branch that lead to the cluster that included the patient's viral sequences. tMRCA for patient 1 was estimated between 1991-2002 (gag) and 1987-2000 (env), consistent with year of diagnosis (1995). tMRCA for patient 2 was estimated between 1990-1999 (gag) and 1985-1996 (env), at least 5 years before diagnosis. tMRCA for patient 3, diagnosed in 2004, was between 1999-2003 (gag), also 1-5 years before diagnosis.

Conclusions: Herein, we show a method that allowed to successfully discard HIV-1 transmission by one man to his ex-wife, who sued him for infecting her; and was consistent with the apriori knowledge that this same man infected his current wife. Furthermore, tMRCA estimates for time of infection were consistent with the date of diagnosis for all patients.

No conflict of interest

Abstract: O_3

Viral Evolution & Genetic Diversity

Preferential suppression of CXCR4-tropic HIV-1 under cART?

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Background: Detection of CXCR4-tropic (X4-) free HIV correlates with CD4 cell deterioration and rapidly progressing HIV infection but little is known about the role of the tropism of integrated provirus. It has been observed that >25% of patients under cART do not achieve a continuous CD4 gain despite full viral suppression, and we find clear evidence that X4-tropic HIV prior to cArt correlates with a later impaired CD4 response. Aim of this study was to follow the evolution of the proviral tropism in infected cells under suppressive cART.

Methodology: Paired PBMC samples from 44 patients (57 patients with paired plasma/PBMC samples) in the Swiss HIV Cohort Study were analyzed at cART initiation and after 5 years of virological suppression. All patients virus was suppressed to <50c/mL. Proviral HIV tropism was assessed with geno2pheno (FPR 5%).

Results The majority of patients presented with R5-tropic proviruses (89%; 39/44) at baseline (BL). 85% of them still had a R5-tropic provirus five years later; in six patients HIV tropism had changed to CXCR4.
When we correlated the tropism of free virus at BL with the later proviral samples on a larger set of 57 plasma/provirus-paired samples we also observed a tight correlation: R5-tropic virus at BL was identified in 49 of 57 patient samples (86%), and 86% had this tropism also five years later. We had not expected to find the ratio to be so similar to the provirus/provirus comparison; but the good correlation suggests that it is appropriate to compare information from virus at BL with later proviral samples from cells.
In surprising contrast the majority of our 13 patients having BL X4-tropic HIV in plasma or provirus presented with R5-tropic provirus five years later (10/13); one patient with X4-tropic HIV at both times was heterozygous for CCR5 Δ32.
In order to address the question of emerging or newly evolving virus: For all tropism conversion cases with BL X4-tropic virus the change to R5-tropic virus was characterized by a number of nucleotide differences in the V3 region needed for the tropism change. This speaks against a continuous and stepwise evolution of the new R5-tropic variants from X4-tropic ancestors. We rather assume an emergence from pre-existing viral minorities. In sharp contrast the tropism change from CCR5 to CXCR4 was in all cases based on single point mutations at positions 11, 24/25 or in a potential glycosylation site in the V3 loop.

Conclusions: Our study provides first evidence that under successful cART a deposition of R5-tropic HIV variants may be favored. This observation is compatible with the hypothesis that immune functions selectively drive the elimination of X4-tropic HIV and of cells potentially expressing X4-tropic HIV envelope. If confirmed in a larger study, these data suggest that strengthened immune function through an early therapy start might be beneficial for virus control. It further provides evidence for the possibility to functionally suppress X4-tropic virus and provirus.
Our data furthermore suggest that proviral testing does not underestimate X4-tropic viruses in the cellular compartment. Larger studies are under way.

No conflict of interest

Abstract: O_4

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Prevalence of resistance mutations associated with decreased susceptibility to rilpivirine in antiretroviral naïve patients from Europe

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Objectives: Current European guidelines recommend second generation NNRTI rilpivirine as one of the preferred first line drugs next to first generation NNRTI efavirenz. We determined the baseline prevalence of mutations that may be associated with decreased susceptibility to first and second generation NNRTIs in antiretroviral naïve patients across Europe.

Methods: The dataset included HIV-1 genotypes obtained in 2002-2010 within 6 months of HIV-1 diagnosis from 8711 therapy-naïve patients from 26 European countries. The prevalence of baseline drug resistance mutations was determined with use of the 2009 epidemiological list of WHO and the 2013 clinical IAS-USA figures. Susceptibility to drugs was predicted with Stanford algorithm v6.3.1 and a genotypic susceptibility score (GSS) was computed for recommended first line regimens (scoring high level resistance as 0, low level/intermediate resistance as 0.5 and potential low level resistance or susceptible as 1).

Results: Based on the WHO list, the prevalence of mutations associated with resistance to NNRTIs remained stable over time: 2.6%, 3.5% and 3.3% in 2002-2005, 2006-2007 and 2008-2010 respectively. A higher prevalence was observed when using the IAS-USA table: 6.6%, 8.4% and 7.8% in 2002-2005, 2006-2007 and 2008-2010 respectively. This difference is mainly caused by inclusion of mutations at position 138 in the IAS-USA table. In 2008-2010, we observed 3.5% E138A, 0.3% E138K, 0.3% E138G, all of which mostly as single mutation. Other NNRTI mutations that were detected include K103N (1.8%), Y181C (0.5%), G190A (0.3%) and H221Y (0.3%). Recently, the Stanford (v6.3.1) and REGA (v9.1.0) algorithms changed the scoring of polymorphic mutation E138A from susceptible to low level or intermediate resistance to rilpivirine. Use of the most recent Stanford algorithm resulted in 0.4% high level resistance and 5% intermediate or low level resistance to rilpivirine in antiretroviral naïve patients. For efavirenz, 2.3% high level resistance and 1.2% intermediate or low level resistance was observed. 7% of patients would have a GSS <3 for a first line regimen of rilpivirine in combination with TDF/FTC or ABC/3TC. For a

regimen that includes efavirenz instead of rilpivirine, 5% of patients would have a GSS <3. Ignoring E138 variants as relevant mutation would result in only 2% resistance to rilpivirine.

Conclusion: E138A is the most frequently observed NNRTI mutation in antiretroviral naïve patients, which is associated with decreased susceptibility to rilpivirine *in vitro*. Considering the recommended use of rilpivirine in first line regimens and the limited availability of therapy outcome data of rilpivirine in patients infected with a virus that harbours E138A, the clinical relevance of this mutation needs to be further investigated.

No conflict of interest

Abstract: O_5

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Reliability and clinical relevance of HCV sequencing on clinical samples with different HCV-RNA levels

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Background: HCV-1a and HCV-1b subtypes have shown different responses to direct antiviral agents (DAA)-based regimens and different prevalence of resistance mutations. Therefore, a correct determination of HCV-subtype is relevant prior to treatment initiation. This study aims to evaluate the reliability and performance of full-length NS3-protease, NS5A and NS5B sequencing to analyze HCV-genotype and presence of drug-resistance.

Materials & Methods: Genotyping success rate (GSR) was evaluated by using a home-made sequencing protocol for full-length NS3-protease, NS5A and NS5B, specific designed for subtype 1a and 1b. Samples were stratified in 6 groups according to different HCV-RNA levels: below the lower limit of detection (<15IU/ml), ≤2, 2.1-3, 3.1-4, 4.1-6, and >6logIU/ml. Phylogenetic analysis was performed to test reliability and reproducibility of genotyping assay and to evaluate concordance with previous subtype assignment by commercial Real-Time PCR approaches.

Results: A total of 656 HCV plasma samples from 219 HCV-infected patients candidate to a new-regimen were collected between 2011-2013: 178 samples had a HCV-RNA undetectable, 85 samples had HCV-RNA ≤ 2 logIU/ml, and 393 had HCV-RNA >2 logIU/ml. Overall, in 478 samples with detectable HCV-RNA, GSR was of 76.1%. In particular, it was 99.2% for samples with HCV-RNA >6 logIU/ml, 98.7% and 92.0% for those with HCV-RNA between 4.1-6 logIU/ml and 3.1-4 logIU/ml, and decreased to 55.2% for samples with HCV-RNA of 2.1-3 logIU/ml and to 4.7% with HCV-RNA ≤2 logIU/ml. Sequences belonging to the same subject (regardless of HCV-RNA level)

clustered always together by phylogenetic analysis.
NS3-sequencing confirmed the infection by HCV-1 genotype in all cases analyzed. Infection by subtype 1b was demonstrated in 214/364 (58.8%) amplified samples, while 147/364 (40.4%) samples were subtype 1a. Notably, 3 samples were collected from one patient infected with an HCV-1 subtype 1g, previously classified as subtype 1a by two commercial Real-Time assays. Overall, subtype assignment was concordant with previous results by commercial Real-Time assays in 98.9% of cases. In 3 patients with previous undetermined subtype by commercial assays (generic HCV-1 in three cases and HCV-1a/1c in the fourth), phylogenetic analysis of both NS3 and NS5A sequences provided the correct subtype assignment (2 were infected with subtype 1b and 2 with subtype 1a). Contextual phylogenetic analysis of NS5A (N=46) and NS5B (N=15) sequences always confirmed subtyping results obtained on NS3-protease sequences.
PI-resistance mutations were overall detected in 103/364 (28.3%) samples by NS3-protease sequencing. This prevalence changed according to PI-experience (from 15.5% in PI-naïve patients to 89% in boceprevir/telaprevir-failing patients). Similarly resistance associated mutations (major and minor) were found also in NS5A and NS5B genes in DAA-naïve patients (39% and 40%, respectively).

Conclusions: Our results suggest that HCV genotypic resistance test provides reliable and reproducible results. In the context of a DAA-regimen, HCV sequencing is an efficient assay that can provide at the same time two important virological information: (1) a correct subtype assignment, and (2) the detection of variants that are potential non responders to therapy (by natural resistance or by previous failure resistance).

No conflict of interest

Abstract: O_6

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

HCV genetic background and early detection of resistance mutations are associated with low viral decay and viral failure to first generation protease inhibitors in difficult-to-treat patients

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Background. Triple-therapy with telaprevir/boceprevir+peg-IFN+RBV can achieve excellent results in terms of antiviral efficacy, but it can be burdened by failures through development of resistance mutations (RAVs). Aims of this study were to correlate pre-existence of novel and known RAVs with treatment-outcome, and to define timing of NS3-mutations development under triple-therapy.

Materials & Methods. 125 patients (GT-1a/1b=46/79; previously non-responders/ relapsers/naive=75/31/20; cirrhotic=61[48.4%]) received telaprevir/ boceprevir+pegIFN+RBV. Baseline (N=112), early time-points (48h-week2-week4; N=29) and failure (N=26) NS3-sequences were obtained by population-sequencing. Baseline ultra-deep-454-pyrosequencing (UDPS) was also performed (N=35).

Results. Virological failure was observed in 31/125 (24.8%) patients (15=GT-1a; 22=previously non-responders). At baseline, a median[IQR] of 5[4-6] NS3 amino-acid variations were detected per patient, compared to wild-type reference-strain. GT-1b failing-patients did not differ for type or number of baseline-mutations compared to patients who reached End-Of-Treatment. A different result was observed for patients with GT-1a. Indeed, failed the PI-treatment: 2/2 (100%) GT-1a patients with baseline boceprevir/telaprevir RAVs (1=V36L; 1=T54S), 4/5 (80.0%) patients with baseline Q80K RAV, and only 8/39 (20.5%) patients without known baseline RAVs (p=0.002 by Chi2 test for trend). Notably, 6/7 GT-1a patients carrying at baseline the novel-mutation P67S failed triple therapy (5 telaprevir, 1 boceprevir). P67S was the only baseline-mutation significantly associated with HCV-RNA>100 IU/ml at triple-therapy week-2 (4/5 vs. 12/41 without P67S, p=0.025). By co-variation analysis, P67S was linked with N174G (p=0.01, Phy=0.470), and 3/3 patients with P67S+N174G failed treatment. P67S and N174G were never found in GT-1b. By UDPS, in the overall population analyzed no additional baseline-RAVs were found (>1% prevalence). The baseline/early detection of NS3-RAVs significantly increased the risk of virological-failure to triple-therapy (OR[CI]= 4.632[1.247-17.213], p=0.022), while baseline detection alone was not predictive (OR[CI]= 2.857[0.619-13.184], p=0.178). At early time-points (48h-2w after treatment start) de-novo development of RAVs was observed in 5/15 failing-patients (V36M; R155T/R+A156A/V; V36M/V+R155K; A156T; R155R/K) vs. only 1/14 End-Of-Treatment patient (T54A/T). This single patient with de novo T54A/T at 48h after telaprevir-treatment, who reached End-Of-Treatment, had a baseline-to-2w HCV-RNA decay similar to all other End-Of-Treatment patients (-4.7 logIU/ml and mean[SE]=-4.4[0.6] logIU/ml, respectively). On the contrary, failing patients with de novo RAVs development had the lowest baseline-to-2w HCV-RNA decay (mean[SE]= -2.5[1.9] logIU/ml), lower than failing patients either without de novo development (mean[SE]= -4.6[0.7] logIU/ml) or without baseline RAVs (mean[SE]= -3.7[0.69] logIU/ml). Overall, baseline-to-2w HCV-RNA decay was significantly lower in patients experiencing virological failure than in those reaching End-Of-Treatment (median [IQR] = -3.7[-4.3;-3.2] vs.-4.6[-5.2;-4.0] logIU/ml, p=0.007).

Conclusions. In the era of direct antiviral agents, particularly in difficult-to-treat patients, HCV-RNA determinations at early time-points, along with the type and kinetics of RAVs detection, may help in rapidly identify patients with higher risk of viral failure and thus requiring a closer monitoring during treatment.

No conflict of interest

Abstract: O_7

Therapeutic Challenges in Resource-limited settings

Increasing prevalence of protease inhibitor resistance mutations in South African adults failing a boosted PI-based regimen

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Background: The South African antiretroviral treatment (cART) guidelines recommend switching adults and adolescents failing a NNRTI-based 1st-line regimen to an empiric PI-based 2nd-line regimen. This study investigated the antiretroviral drug resistance profiles in patients failing a PI-based 2nd-line regimen attending an urban referral clinic in Johannesburg. Previous data, collected between 2009 and 2010 from the same region, showed a high prevalence of complete susceptible drug resistance profiles (38.7%) and only 6.7% PI-resistance.

Methods: A retrospective dataset of 58 HIV-1 drug resistance results from adults attending the HIV clinic at Charlotte Maxeke Johannesburg Hospital, and failing a PI-based regimen at the time of testing (between 2012 and 2013), was analysed. Data was extracted and *pol* sequences were resubmitted to the Stanford HIV database (v6.3.1) for genotypic predictions.

Results: Most patients were female (62.1%) with a median age of 38 years. Patients presented with a median VL of 4.2 $\log_{10}$ RNA copies/ml and a median CD4-count of 254 cells/mm^3. Patients had been treated with cART for a median of 61 months (range 18-105 months) with a median exposure time to PIs of 29 months (range 4-99 months). Three patients had been exposed to boosted atazanavir in addition to the standard boosted lopinavir. Phylogenetic analysis revealed two patients were infected with HIV-1 subtype B and D and the remainder with HIV-1 subtype C (96.6%).
Ten patients (n= 17.2%) failing a PI-based regimen did not show any resistance to PIs, NRTIs or NNRTIs suggesting non-adherence to treatment. Intermediate resistance to a single drug was detected in five patients, attributed to non-B specific polymorphisms: T74S (n=3), causing intermediate resistance to nelfinavir and E138A (n=2) causing intermediate resistance to etravirine. This brings the suspected level of complete non-adherence to 25.9% compared to 38.7% previously reported (p=0.1394).
Fourteen patients (24.1%) presented with at least one major PI-mutation, which is significantly higher compared to the previously reported 6.7% (p=0.0057). The most common PI-mutations were I54V (n=11), V82A (n=10) and M46I/L (n=9). A combination of three or more major PI-mutations was detected in nine patients. Two patients presented with intermediate resistance to four PIs, caused by a single mutation (I54V or V82A). One patient showed PI resistance alone, whereas another showed PI and NRTI resistance. The remaining 10 patients (17.3%) presented with extensive triple class resistance, after a median of 45 months of PI-exposure. However, all 10 patients remained susceptible or partially susceptible to boosted darunavir, etravirine and rilpivirine.

Conclusion: Analysis reveals the prevalence of fully susceptible drug resistance profiles, likely attributed to poor adherence, is decreasing in this population over time. By contrast, the frequency of patients presenting with some level of PI -resistance is increasing. The increasing presence of triple-class resistance in patients presenting with 2nd-line virological failure justifies the introduction of routine HIV antiretroviral drug resistance testing in these patients. Most patients with triple-class resistance will benefit from an individualised regimen containing a combination of raltegravir, boosted darunavir and etravirine, now available in the South African public health sector.

No conflict of interest

Abstract: O_8

Novel Diagnostic Technologies & Approaches

One year of routine HIV-1 drug resistance testing by deep sequencing: insights from comparative Sanger sequencing

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Background: HIV-1 drug resistance testing is routinely performed prior to antiretroviral treatment initiation or resistance-related therapy changes. Genotypic tests are mainly based on population Sanger-sequencing considering variants with frequencies above 20% of the whole viral population. Next generation sequencing technologies enable deep sequencing strategies with detection sensitivities of 1% and below. However, thus far these strategies have not yet entered the daily routine in a widespread manner.
Here, we present data of more than 400 samples, analysed with both population and deep sequencing in daily routine to obtain insights in sensitivity, accuracy and the putative impact of minority variants on resistance interpretation.

Material & Methods: Population (PS) and deep sequencing (DS) on protease/reverse transcriptase (PR/RT), integrase (INT) and gp120 (V3) were performed from specimen of therapy-naïve and therapy-experienced patients for whom genotypic drug resistance testing was requested in 2013.
Amplification and PS were done according to homebrew protocols. For DS, the same PCR products were used for Nextera XT® library preparation. DS was done using Illumina's MiSeq benchtop sequencing system.
All bioinformatics analyses were performed using an automated customized pipeline. Resistance mutations were defined according to Stanford HIVdb. Minority frequencies were analysed using cutoffs at 20%, 15%, 10%, 5%, 2%, and 1%. Drug resistance interpretations were computed using the HIV-GRADE algorithm and geno2pheno[454] for tropism predictions. DS-R5-tropism was defined as <2% X4 at FPR of 3.5%.

Results: Paired PS and DS data were available for 312 PR/RT, 23 INT, and 97 V3 samples. The median DS-coverages were 29655 (PR/RT), 15437 (INT), and 39571 (V3). In total, 236 resistance mutations were detected by PS similar to the detection cutoffs at 20% (230) and 15% (239). For a 10%, 5%, 2%, 1% cutoff, 252, 275, 324, and 499 mutations were detected, respectively. By applying a 2% minority cutoff, discrepancies were most obvious for PIs (65 DS vs. 24 PS mutations), followed by the NRTIs (162/131), and NNRTIs (97/81). The most frequent NNRTI mutation K103N occurred in 63 (DS) and 61 samples (PS), respectively, indicating a higher sensitivity of PS at this position. INT-PS detected 4 resistance mutations in total, whilst the respective DS numbers were 8 (20% cutoff), 8 (15%), 10 (10%), 10 (5%), 12 (2%), and 13 (1%).
Depending on the DS cutoff used, significant differences in the interpretation of the resistance situation were observed relative to the PS-based interpretation.
DS determined 26 samples to be X4, whereas PS found 28 X4 at the 10% FPR level (20 at 5% FPR). PS-X4 samples that were DS-R5 had FPRs of >7.5. There was a very high correlation between the frequencies of X4 DS-reads and the FPR of X4-PS samples (r-squared=0.85).

Conclusions: DS is a powerful tool for routine drug resistance testing in therapy-naïve and therapy-experienced patients. Applying a generalized „PS-derived' cutoff of 15-20% to DS data may result in the non-consideration of clinically relevant mutations. Thus, there is an urgent need to determine meaningful clinical cutoffs for drug resistance interpretation.

No conflict of interest

Abstract: O_9

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Reliability of HIV-1 Integrase genotyping in patients with low viremia levels

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Background: Genotyping testing for resistance to integrase inhibitors (INI) is suggested in drug-naïve patients, and recommended in patients who had failed this class of antiretrovirals. Its clinical value may be more relevant if the diagnosis of virological failure is done at early time points, that is when viremia is still contained.
Aim of this work was then to evaluate the reliability and usefulness of HIV-1 integrase genotyping in samples with low levels of viremia (LLV).

Materials & Methods: A retrospective analysis was performed on 1,734 HIV-1 clinical samples that were genotyped over the years 2004-2013 in two clinical centers in Rome, Italy, by means of a homemade system or the commercially available ViroSeq HIV-1 Genotyping System (Abbott Molecular). For each clinical sample, the viremia value at genotyping was known. The retrieved 1,734 samples were stratified in 6 groups according to different viremia levels (51-200; 201-500; 501-1,000; 1001-10,000; 10,001-100,000, >100,000 copies/mL). Genotyping success rate (GSR) was determined in total and per each viremia-group, independent of the genotyping platform (equipment, kits and reagents) upgrades that occurred during this period. Phylogenetic analysis was performed to test the reliability and reproducibility of the integrase genotyping also at LLV. Finally, the relationship between different viremia levels at the time of the integrase genotyping and the detection of raltegravir resistance mutations (IAS-Stanford list 2013) was evaluated in a subset of 170 samples successfully genotyped from 129 patients who had failed a regimen containing raltegravir (virological failure defined as viremia >50 copies/mL). Positive and statistically significant correlations among the raltegravir resistance mutations were defined in this set of 170 samples.

Results: Overall, GSR of amplification/sequencing was 95.7%. Viremia-levels of 51-200 and 201-500 copies/mL afforded success rates of 81.3% and 83.6%, respectively, reaching 94.0% at 501-1000 copies/mL and Ž94.9% above 1000 copies/mL. GSR was comparable in the two centers where the sequences were produced (95.4% vs. 96%, p=0.542). Phylogenetic analysis revealed a high homology among sequences belonging to the same subject for 97.6% of patients analyzed. The overall raltegravir resistance prevalence at raltegravir failure was 32.9%. Drug-resistance was found also at LLV. Detection of at least one raltegravir major resistance mutation was indeed: 51-200 copies/mL=10.3%; 201-500=33.3%; 501-1000=35.3%; 1001-10000=52.4%; 10001-100000=29.3%; >100000=30.8% (p=0.107). The following significantly correlated pairs of raltegravir-mutations were found: N155H+V151I (covariation-frequency=76.9%; phi=0.46; p<0.001); Q148H/K/R+G140S (covariation-frequency=100%; phi=0.82; p<0.001); Y143C/H/R+S230R (covariation-frequency=100%; phi=0.47; p=0.021); Y143C/H/R+T97A (covariation-frequency=40.8%; phi=0.37; p=0.026). Interestingly, at viremia levels <200 copies/mL all samples with resistance contained only one raltegravir mutation, with the exception of one sample where the combination N155H+V151I was found.

Conclusions: Reliable and reproducible HIV-1 integrase genotypes can be obtained from plasma samples with a high rate of success also in patients with viremia levels in the range of 51-1,000 copies/mL (>81%). These results are helpful for a correct management of antiretroviral therapy because they provide

information on the early emergence of drug-resistance also at low levels of viremia.

No conflict of interest

Abstract: O_10

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Durable efficacy and limited Integrase resistance in subjects receiving Dolutegravir after failing a prior INI regimen: Week 48 results from VIKING-3

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Background VIKING-3 examined the efficacy and safety of dolutegravir (DTG) 50mg twice daily with 7 days functional monotherapy followed by optimization of background ART in patients with resistance to multiple antiretrovirals, including integrase inhibitors(INIs). Here we summarize efficacy through Week 48 and treatment-emergent (TE) resistance detected at protocol-defined virological failure (PDVF).

Methods Efficacy was assessed as proportion of subjects with HIV-1 RNA<50 c/mL at Week 48 (ITT-E population, Snapshot algorithm). TE resistance was defined as INI mutations detected at PDVF timepoint but not at Baseline. PDVF was defined as any confirmed HIV-1 RNA value >400 c/mL and meeting following criteria: <0.5 $\log_{10}$ decrease at Day 8, or confirmed decrease of < 1 $\log_{10}$ c/mL by Week 16 or confirmed >1 $\log_{10}$ c/mL above a nadir of ≥400 c/mL, or detected on or after Week 24 or after prior confirmed <400 c/mL.

Results Through Week 48, 63% (116/183) of subjects achieved HIV-1 RNA <50 c/mL, demonstrating a sustained response from that seen at Week 24 (69% with HIV-1 RNA <50 c/mL). Week 48 responses were associated with Baseline integrase (IN) genotype: 71% (90/126) of subjects with No Q148 mutation achieved HIV-1 RNA <50 c/mL, and for subjects with Q148 + 1 and Q148 +≥2 additional secondary mutations (amongst L74, E138, and G140) 56% (20/36) and 29% (6/21), respectively, achieved <50 c/mL. At Weeks 24 and 48, 20% and 22% of subjects met PDVF, with an additional 4 subjects identified after Week 48 (25%, 45/183). Of the 45 subjects, 56% (22/39) with paired Baseline and PDVF samples had TE mutations detected at PDVF. The TE mutations detected for these 22 were seen at the following positions (with # subjects): L74M/I (3), E92Q (2), T97A (10), E138K/A/T (9), G140S (4), Y143H (1), S147G (1), Q148H/K (6), N155H (4), E157Q (1). Of these 22 subjects with TE resistance detected, 18/22 harboured virus with a mutation at Q148 at Baseline, at Screening or historically. For 39 subjects with paired IN phenotypic data at baseline and PDVF, 18/39 had an increase in DTG FC; TE mutations were seen in all 18.

Conclusions Antiviral response to DTG 50 mg BID in subjects with INI resistance was sustained through Week 48, with only a 2% increase in PDVF between Weeks 24 and 48. Limited viral evolution was detected at PDVF, comprising previously identified INI mutations, which were most commonly added to virus harbouring Q148 mutations.

Conflict of interest: Employee of GlaxoSmithKline

Abstract: O_11

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Clinical consequences of Raltegravir failure in Spain

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Background: Clinical consequences of virological failure to integrase inhibitor (INI)-based ART are unknown.

Methodology: This was a multicenter observational retrospective study including all HIV-1-infected subjects receiving raltegravir (RAL) or elvitegravir (ELV) in routine clinical care in 10 Spanish hospitals between June 2005 and June 2013. Two consecutive HIV-1 VL ≥200 c/mL 24 weeks after INI initiation was considered virological failure (VF), and low-level viremia (LLV) was defined as 2 consecutive VL between 50 and 200 c/mL. Antiretroviral drug resistance at INI failure was defined using the HIVdb algorithm. To account for recent publications, dolutegravir (DTG) resistance was defined by adding the following scores to the DTG HIVdb rules available on June 4th 2013: 20 points to each combination of mutations 148HRK+74I and 148HRK+138T, and 5 points to mutation 74I. Virological, immunological and clinical outcomes during the 1st year of salvage ART following INI failure were collected. Logistic regression analysis was performed to evaluate factors associated with VF of the salvage ART initiated after INI failure.

Results: From a total of 15,009 HIV-treated subjects, 2,799 (18.6%) had received RAL and none ELV. Of these, 209 (7.5%) subjects had VF or LLV. RAL regimens had been initiated as first-line ART in 8 (3.8%) subjects, following VF in 118 (56.5%), as a switch strategy in 71 (33.9%), after an ART interruption in 5 (2.4%), and due to other reasons in 7 (3.3%) individuals. The prevalence of VF and LLV to RAL treatment were 4.93% (95% CI: 4.16, 5.79) and 2.53 % (95% CI: 1.99, 3.19), respectively. Only 33 (15.8%) integrase genotypes were available from subjects with VF or LLV. INI-associated mutations were detected in 26/33 (78.8%). Most frequent mutations were 155H (36.4%); 140S (18.2%); 148H (15.2%); 143R, 151I, and 203M (12.1%); 148R (9.1%); and 74M, 92Q, and 143C (6.1%). The most common combinations of mutations were: 155H +1 (24.2%); 148HKR +1 (15.2%); 148HR +140S + ≥ 1 (18.2%); 148HKR + ≥ 2 (9.1%); and 155H + ≥ 2 (9.1%). High-level resistance to RAL and ELV was predicted in 17 (51.5%) and 15 (45.5%) cases, respectively. Full susceptibility to DTG was predicted in 22 (66.7%) cases. No cases of high-level resistance to DTG were observed. Of the 192 subjects with RAL VF or LLV, 10 (5.2%) experienced AIDS events and 10 (5.2%) died during the following year after. In the multivariate analysis, factors associated with VF of the salvage ART following INI failure were: baseline and increased CD4 cells, self-reported adherence <90%, and maintaining use of RAL and boosted PI in salvage regimen.

Conclusions: Virological failure to RAL is infrequent in Spain, but it can lead to major clinical complications in ART-experienced subjects. Rates of integrase resistance genotyping at raltegravir failure are low. DTG remains as a suitable drug for salvage ART in most RAL failures.

Conflict of interest: This work was supported in part by Lluita contra la SIDA Foundation (Barcelona, Spain) and ViiV Healthcare (Tres Cantos, Spain)

Abstract: O_12

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Longitudinal resistance analyses of the phase 3 EVG/COBI/FTC/TDF studies

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Introduction: In Phase 3 studies, Week 144 treatment responses in treatment-naïve subjects were durable and similar for EVG/COBI/FTC/TDF vs. efavirenz (EFV)/FTC/TDF) (82% vs. 78%; GS-US-236-0102) and EVG/COBI/FTC/TDF vs. ritonavir-boosted atazanavir (ATV+RTV) + FTC/TDF (81% vs. 79%; GS-US-236-0103). Emergent resistance to EVG, FTC, or TDF at the virologic failure confirmation visit was infrequent through Week 144. The aim of this study was to understand the order and timing of mutation development in subjects with emergent resistance from the EVG/COBI/FTC/TDF groups.

Methods: HIV-1 genotypes (protease and RT) were analyzed at screening. The resistance analysis population (RAP) consisted of subjects with suboptimal virologic response (< 1 $\log_{10}$ reduction in HIV-1 RNA at Week 8 confirmed at Week 12), virologic rebound (confirmed HIV-1 RNA ≥ 400 copies/mL after achieving HIV-1 RNA < 50 copies/mL or confirmed ≥ 1 $\log_{10}$ rebound from nadir), or a single visit with HIV-1 RNA ≥ 400 copies/mL at Week 48, 96, 144, or the last visit (discontinuation for any reason). Subjects in the RAP had genotypic and phenotypic analyses of RT, protease, and integrase at the failure confirmation visit and at baseline. The first failure analysis consisted of genotyping of samples from the first time point of virologic failure with HIV-1 RNA ≥ 400 copies/mL from the 18 subjects with known emergent resistance. Repeat samples and alternative assays were utilized to generate datasets that were as complete as possible. All resistance analyses used population sequencing and were conducted at Monogram Biosciences or Labcorp.

Results: In the EVG/COBI/FTC/TDF group through Week 144, 18 subjects (2.6%; 18/701) developed primary integrase strand transfer inhibitor (INSTI) (N=15) and/or nucleoside/tide reverse transcriptase inhibitor (NRTI) resistance mutations (N=17). Of these 18, 14 had both NRTI and INSTI resistance mutations at the confirmation visit. The first failure samples from these subjects were obtained a median of 30 days prior to the confirmation visit (range 7 to 220 days). The visit dates for the first failure samples were prior to Week 24 for 5 subjects and at or after Week 24 for 13 subjects. At first failure, mutations were not detected for 2 (12%) of the subjects with later NRTI resistance mutations (primarily M184V/I) and 7 (47%) of the subjects with later INSTI resistance mutations. Overall, the first failure sample detected only 29 (57%) of the total 51 mutations in RT and integrase observed in the confirmatory sample.

Conclusions: Treatment with EVG/COBI/FTC/TDF achieved durable high rates of virologic suppression in HIV-1 treatment-naïve subjects with infrequent resistance development. Longitudinal analyses found that the emergence of the M184V/I in RT often precedes emergence of resistance mutations in integrase. Analysis of samples obtained at first failure found fewer mutations overall and failed to detect mutations in IN in half those subjects with mutations at the confirmation visit.

Conflict of interest: Employee and stock holder of Gilead Sciences, Inc.

12th European Workshop on HIV & Hepatitis

26 – 28 March 2014, Barcelona, Spain

Abstracts

Poster presentations

Abstract: P_1

Viral Evolution & Genetic Diversity

Phylogenetic analysis of Hepatitis Delta Virus isolates from HBsAg positive patients in Karachi, Pakistan

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Background: In spite of a high occurrence of Hepatitis Delta infection in the province of Sindh in Pakistan, no genetic study of Hepatitis Delta virus (HDV) isolates from this region was carried out. The aim of this study is to analyze the genetic proximity within local HDV strains, and relationship with other clades of HDV, using phylogenetic analysis.

Material and Methods: About 5cc whole blood samples were collected from 22 patients diagnosed with chronic hepatitis delta infection. Serum was separated and viral RNA was extracted from 140µl serum. cDNA was used to amplify HDV R0 region by RT-Nested PCR. PCR products were screened with RFLP. After nucleotide sequencing of HDV R0 region the maximum likelihood and Bayesian phylogenetic analysis under the nucleotide substitution model were conducted for molecular epidemiology analysis of HDV isolates followed by aminoacid sequence analysis to check atypical amino acid substitution within our HDV isolates.

Results: Phylogenetic analysis of nucleotide sequences of the Hepatitis Delta Antigen (HDAg) R0 region obtained in this study, showed considerable diversity among the local strains with a potential subgroup formation within clade I. This potential subgroup was therefore more distant from most clade I sequences, but interestingly was more close to the clade I sequences from geographically distant areas (Italy and USA).The multiple sequence alignment of predicted amino acids within clade I showed many uncommon amino acid substitutions within some conserved regions that are crucial for replication and assembly of HDV.

Conclusions: The studied strains showed a range of genetic diversity within HDV clade I. There is clustering of sequences into more than one group, along with formation of potential subgroup within clade I. Clustering shows the genetic closeness of strains and indicates a common origin of spread of HDV infection. Further phylogeny-based studies may provide more information about subgroup formation within clade I and may be used as an effective tool in checking and/or preventing the spread of hepatitis D virus infection in this region.

No conflict of interest

Abstract: P_2

Viral Evolution & Genetic Diversity

The characteristics of the HIV subtype B epidemic in Slovenia

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Background: Slovenia is a small Central European country with a relatively modest burden of HIV disease, with fewer than 1 per 1000 inhabitants infected. The HIV epidemic mostly affects men who have sex with men (MSM), with subtype B as the most represented subtype in over 85% of patients. The aim of this study was to establish the properties of the subtype B HIV epidemic in Slovenia up to the end of 2012.

Materials & Methods: For the purpose of this study, data and sequences were gathered from

3 previous studies conducted in Slovenia examining the prevalence of transmitted drug resistance among therapy naive HIV-1 positive patients diagnosed in the years 2000-2012. Sequences were re-analyzed for subtype determination by employing the REGA HIV-1 Automated Subtyping Tool, version 2. Only subtype B sequences were selected for this study, a total of 223 partial *pol* gene sequences were included, representing 52% of all patients newly diagnosed in 13 years.
First jModeltest software was employed for selection of the best fitted evolutionary model to be used on the selected dataset of 307 subtype B sequences (Slovenian sequences with control sequences selected from GenBank). The maximum likelihood (ML) phylogenetic tree was constructed by using PhyML 3.0 and transmission clusters were identified according to the approximate likelihood ratio test (aLRT) branch support values obtained (>0.90).
The Monte Carlo Markov chain (MCMC) method available in the BEAST package v1.7.1 was employed, using a relaxed clock model with uncorrelated lognormal distribution and the Bayesian skyline coalescent model. All the analyses were repeated and the obtained duplicate results combined using LogCombiner v1.6.1, available in the BEAST package. The output results of BEAST were examined in Tracer v1.5 and the length of the chain was elongated if the effective sample size (ESS) values did not exceed 200. TreeAnnotator v1.7.1 was employed to annotate the obtained tree files with 10% burnin. The clusters previously identified in the ML analysis were reviewed and confirmed according to posterior probability values (>0.990).
Statistical analyses were conducted using the on-line statistical package Epi Info™ Version 3.5.3 and P≤0.05 values were considered to be significant.

Results: Combined analysis (ML and Bayesian analysis) revealed 8 major clusters (n≥10 patients), 1 group of 4 patients, 2 trios and 12 transmission pairs. Among 223 included individuals, 146 (65.5%) patients belonged to large transmission clusters comprising 10 or more individuals and 34 (15.2%) patients to small clusters of 2-4 patients, leaving only 43 (19.3%) of the Slovenian patients infected with subtype B without an epidemiological link observed by phylogenetic inference. Statistical analysis examining the characteristics of patients found in large clusters revealed significantly fewer patients in a cluster diagnosed prior to 2005 (p=0.0388) and significantly more patients reported Slovenia as the country in which the infection occurred (p<0.0001) than reported other countries.

Conclusions: In conclusion, several introductions of HIV subtype B occurred in Slovenia. The majority of patients were found with a transmission link, exhibiting a closed HIV community, with the virus being transmitted predominantly between individuals within Slovenia.

No conflict of interest

Abstract: P_3

Viral Evolution & Genetic Diversity

Rapid increase in HIV-1 cases among IDUs in Bulgaria is accompanied by the domination of CRF 01_AE and CRF 02_AG and co-infection with hepatitis

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Background: Over a period of seventeen years from 1986 to the end of 2003 only seven cases of injecting drug users (IDUs) with HIV-1 infection were registered in Bulgaria. By 2009, the IDU number rose to 78 and as of 2011, 359 (24.8%) of all 1446 registered HIV/AIDS cases in Bulgaria were IDUs. Nonetheless, limited information is available to provide molecular

insights into the HIV-1 epidemic among IDUs in Bulgaria. The aim of the present study was to determine genetic diversity among IDUs with HIV-1 infection in Bulgaria and to establish the prevalence of HIV-1 and hepatitis B and C co-infections in this group.

Materials and Methods : HIV-1 diversity was determined in 117 (32.6%) of 359 IDU patients diagnosed with HIV/AIDS. HIV-1 polymerase (*pol)* sequences were generated using the TruGene DNA Sequencing System (Siemens Healthcare). The sequence alignment for phylogenetic analyses contained Bulgarian sequences, subtype reference sequences and the most similar sequences found by BLAST searches. 23 codons with resistance mutations were removed from the dataset to exclude possible convergent evolution. Phylogenetic relationships were inferred by maximum likelihood using FastTree2. HIV-1 recombination was investigated using bootscan analysis with SimPlot v3.5. In addition, ELISA testing for hepatitis B surface antigen and anti-hepatitis C antibodies was performed in a subset of patients.

Results: Of the 117 IDU *pol* sequences, 50 (42.7%) were classified as CRF 02_AG, 41 (35%) as CRF 01_AE, ten (8.5%) as subtype B, two (1.7%) as subtype F1 and two (1.7%) as CRF 14_BG. Genotype analysis also classified 12 sequences (10.3%) as unclassified recombinant forms (URFs). Phylogenetic analysis showed that two different circulating recombinant forms (CRFs) were introduced and distributed among IDUs in the two main cities of Bulgaria: Sofia and Plovdiv. 42 (35.9%) samples were from individuals who live in the capital city of Sofia and of these, 73.8% were CRF 01_AE. In contrast, 48/59 (81.4%) patients from Plovdiv were CRF 02_AG. The remaining sequences from Sofia comprised four (9.5%) subtype B, four (9.5%) URF, two (4.8%) CRF 14_BG and only one (2.4%) CRF 02_AG. In Plovdiv, eight (13.6%) were URF, two (3.4%) CRF 01_AE, and one sequence (1.7%) was subtype B. The remaining 15 IDU samples were from other locations, of which eight were CRF 01_AE, five were subtype B and two patients were infected with subtype F1.
24/111 (21.6%) HIV-1-infected persons were positive for HBsAg and 129/142 (90.8%) were HCV positive. 15/83 (18.1%) HIV-1 patients were co-infected with hepatitis B and C.

Conclusions: We found a rapid increase in the prevalence of HIV-1 among IDUs in recent years. Phylogenetic analysis revealed the introduction of two main HIV-1 subtypes in distinct geographic sub-epidemics in two major cities in Bulgaria. Co-infections of HIV-1 with hepatitis B and/or hepatitis C are widespread among IDUs in Bulgaria. Our study provides valuable information on viral infection in IDUs and shows that continued molecular surveillance of IDUs and other high risk groups is key to controlling the spread of these pathogens in Bulgaria.

No conflict of interest

Abstract: P_4

Viral Evolution & Genetic Diversity

HCV molecular epidemiology in HIV/HCV coinfected patients in Serbia

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Introduction/Background: About one quarter of HIV-infected persons in Serbia have also been found to be HCV infected (24,3%). According to the existing data, genotype 1 is the most prevalent HCV genotype in general, non HIV infected, population in Serbia. So far, no data is available about HCV genotype distribution among co-infected patients.

Material & Methods: The study prospectively included 214 HIV/HCV co - infected and a matching group of 193 HCV mono - infected patients in the Center for HIV/AIDS, Institute for Infectious and Tropical Diseases in Belgrade upon following inclusion criteria: ELISA

determined and western blot confirmed seropositive status for HCV monoinfection or HIV/HCV coinfection and adult age. Epidemiological, clinical, and behavioral data were collected using a standardized questionnaire. Transmission risk was defined as intravenous drug use (IDU), sexual, transfusion of blood/blood products, vertical and other. HCV viral load detection and genotyping was performed on 229 samples, using commercial tests.

Results: The prevalence of transmission risk was found to be substantially different among the two groups. Majority of coinfected patients (151/214, 70.5%) were IDUs, compared to 37/193 (19.1%) HCV monoinfected patients (P<0.01); .sexual transmission was reported by 2.5% monoinfected, compared to 22.8% coinfected patients (P<0.01). HCV genotype distribution was also found to be significantly different: genotype 1 was detected in 87% of patient with monoinfection, compared to 16.3% of patients with coinfection (P<0.01); non-1 genotypes, namely genotypes 3 and 4 were significantly more common among coinfected patients (6% and 5%, vs. 27% and 25%, respectively). Non-1 HCV genotypes were more commonly found among IDUs compared to other transmission risks, irrelevant of HIV status. Multivariant logistic regression identified IDU, infection with non-1 HCV genotype and HCV viral load over 5log to be associated with HIV coinfection.

Conclusions: HCV genotype distribution among HIV/HCV coinfected patients in Serbia is substantially different compared to HCV monoinfected patients, with much higher prevalence of non-1 genotypes, in particular genotypes 3 and 4. This difference may be linked to higher prevalence of IDU among the coinfected.

No conflict of interest

Abstract: P_5

Viral Evolution & Genetic Diversity

Evolutionary and epidemic dynamics of HIV in a large Russian data collection

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Background: The Rostov Research Institute (RRI), located in Rostov on Don, has primary responsibility for treating patients in the Southern and the North Caucasian Federal Districts of Russia. Patients include the tragic case of a number of infants nosocomally infected (INI) from a common HIV subtype G source virus in the late 1980s during routine heparin treatment. The outbreak spanned two hospitals, Kalmykia and RRI.

Material and Methods: Longitudinal data from 75 INI includes 191 HIV RT sequences collected over more than 20 years (1991-1995 n=36, 2000-2005 n=59, 2006- 2010 n=65, 2010-2013 n=31). A consensus estimate of the infecting strain is also available, dated at 1990. A maximum likelihood (ML) phylogenetic tree was computed based on these sequences using RAxML under a general time reversible model with a gamma model of rate heterogeneity. Additionally, both inter- and intra-host evolutionary rates were computed from observed sequence divergence over known time intervals. Inter-host rates are based on the earliest sequences from each patient and the infecting strain. Intra-host rates are based on the distance from all later samples to the first sample from each patient, and thus occur after the virus has adopted to the host.

Results: Of the 18 patients sampled four or more times, 15 were monophyletic in the ML

tree. The observed intra-host mean number of substitutions per site per year (ssy) is 0.006, an order of magnitude less than the inter-host mean of 0.023 ssy. Likewise, the ML tree clearly separates patients based on hospital of infection, and the distribution of inter-host ssy values has two distinct peaks (excluding 5 outliers); the first at 0.012 ssy is enriched for patients from RRI (32/45 patients), the second at 0.33 ssy is enriched for Kalmykia (24/25 patients).

Conclusions: HIV evolution in the nosocomally infected infants appears to be an order of magnitude faster between patients than within patients. While intra-host rates were measured after the virus had several years to adopt to the host, the ability of molecular epidemiology to separate patients from the two hospitals suggests that the results generalize to earlier phases of infection.

No conflict of interest

Abstract: P_6

Viral Evolution & Genetic Diversity

Duration of infection estimated from viral diversity: a sequence ambiguity model based on HIV-1 population sequences from the German HIV-1 Seroconverter Cohort

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Background: It was recently reported that viral diversity reflected by the proportion of ambiguous bases in HIV-1 *pol* population sequences is linked to the duration of patient's infection. We established a sequence ambiguity model (SAM) based on 1343 HIV-1 *pol* population sequences derived from longitudinal samples of 475 antiretroviral treatment-naïve patients with known duration of infection up to 13 years. Subsequently, we evaluated the utility of SAM to classify recently acquired infections and compared the performance of SAM and a serological incidence test (BED-CEIA).

Material and Methods: Patients from the German HIV-1 Seroconverter Cohort with at least two available *pol* population sequences were included if i) they had a first reactive test before the immunoblot result was positive or ii) their last negative and first positive antibody test fell ≤365 days apart. Sequences were trimmed to a length of 1170 nucleotides spanning amino acids 1-99 of the protease and 1-291 of the reverse transcriptase. Resistance mutations were identified according to the surveillance drug resistance mutation list. HIV-1 subtypes were determined by the REGA HIV Subtyping Tool (Version 2.0) and by additional phylogenetic analysis (PHYLIP Version 3.6, Joe Felsenstein). Simple and multiple linear regression was used to analyze the correlation between sequence ambiguities and duration of infection. Additionally, 252 samples from 181 seroconverters infected with subtype B were tested in the AWARE^{TM} BED^{TM} EIA HIV-1 incidence test (BED-CEIA; Calypte Biomedical Corporation) performed according to the manufacturer's instructions.

Results: We identified a significant linear increase of 0.20% ($CI_{95\%}$ 0.19-0.22) sequence ambiguities per year of infection. Subtype B (n=1236) and resistant viruses (n=184) had a higher proportion of sequence ambiguities than subtype non-B (n=107) and sensitive (n=1159) viruses. An ambiguity cutoff of 0.50% optimally classified infections acquired ≤520 days with 76.6% (541/706) sensitivity and 76.9% (490/637) specificity. For the comparison of SAM and BED-CEIA, the best accuracy to identify subtype B infections acquired ≤162 days resulted in an ambiguity cutoff of 0.25%. SAM reached 69.3% (88/127) sensitivity and 79.2% (99/125) specificity compared to 76.4% (97/127) sensitivity and 88.8% (111/125) specificity achieved by BED-CEIA. Results of BED-CEIA and SAM were concordant in 72.6% (183/252) of cases. Combining both methods increased the sensitivity to 83.0% (73/88) and the specificity to 94.7% (90/95), corresponding to a false recent rate of only 5.3%.

Conclusions: In conclusion, SAM is not superior to BED-CEIA and not suitable to estimate the HIV-1 incidence in populations. Combining SAM and BED-CEIA substantially improved the false recent rate, but due to the high costs of HIV-1 genotyping SAM is not recommendable for a multiassay algorithm, as well. Nevertheless, SAM allows durations of infection to be differentiated over a longer period of time than possible using serological tests and can therefore provide an additional parameter to characterize study populations in epidemiological resistance studies.

No conflict of interest

Abstract: P_7

Viral Evolution & Genetic Diversity

Re-treatment with telaprevir-based triple therapy after telaprevir exposure in phase 1 studies: deep sequence analysis for persistence of resistant variants

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Background: Potential for persistence of HCV NS3 protease inhibitor-resistant variants at low levels after telaprevir monotherapy and re-selection during a second telaprevir-based therapy is unknown.

Methods: Nine patients who received 14 days' telaprevir monotherapy were re-treated with telaprevir plus peginterferon-alfa (P) and ribavirin (R). In four patients without SVR, NS3 deep sequence analysis (454 GS flx) was performed at baseline before initiation (BL1 and BL2) and at treatment failure of the first and second telaprevir exposure (EOT1 and EOT2). Phylogenetic analysis sought: (i) resistance mutations responsible for first and second viral failure; (ii) linked neutral mutations disclosing descent of resistant strains at EOT2.

Results: Three previous PR non-responders and one relapse patient experienced on-treatment virologic failure (n=2) or relapse (n=2) to therapy. Deep sequence analysis revealed no telaprevir-resistant variants at BL1 and BL2 and isolates with variants at positions 36, 54, 155 or 156 conferring resistance to telaprevir at EOT1 and EOT2 in all patients (Figure 1 example). Based on the number of nucleotide changes in the NS3 backbone sequence, heterogeneous evolution of isolates was visible at different time points. In three patients, dominant isolates were detected at BL2 and EOT2, which were invisible at the previous time point. One patient showed a stepwise evolution of isolates from one time point to the next.

Conclusions: Deep sequence analysis showed a heterogeneous evolution of viral isolates in patients with repeated exposure to telaprevir, including persistence of wild-type and resistant variants at low levels, with reappearance as dominant quasispecies at later time points.

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No conflict of interest

Abstract: P_8

Viral Evolution & Genetic Diversity

Molecular epidemiology analysis of HIV-1 epidemic in the Northwest of Spain: evidence for local transmission of subtype F

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Background: Although subtype B is the prevalent clade in Europe, there are several regions where different subtypes and recombinants are circulating. Interestingly across Europe, subtype F dominates only in Romania. Herein, we analyzed the molecular epidemiological characteristics of the HIV-1 epidemic in two regions of the Northwest of Spain among newly HIV diagnosed patients in the last 5 years.

Material & Methods: A total of 420 newly HIV diagnosed patients during 2009-2013 from two regions of the Northwest of Spain (A Coruña and Santiago de Compostela) were included in this study. HIV-1 strains were genotyped in the protease and reverse transcriptase genes by using TRUGENE® HIV-1 genotyping assay. HIV-1 subtyping was done using automated subtyping tools and phylogenetic analysis. Phylogenetic analysis was performed using fast maximum likelihood as implemented in fasttree, using the GTR (general time reversible) as nucleotide substitution model and gamma (γ) distribution of rate variability among sites. Phylodynamic analysis was performed using Bayesian method as implemented in BEAST.

Results: The most prevalent subtype was subtype B (64.7% and 70.4%) followed by subtype F (25.5% and 12%) in both regions (A Coruña and Santiago de Compostela, respectively). Additionally, we detected at low prevalence subtypes A1, C, G, CRFs and URFs. Notably, subtype F1 was found mainly among men having sex with men (MSM). To estimate the patterns of HIV-1 transmission for both subtypes we performed phylogenetic analysis using a large collection of globally sampled sequences as references. For subtype F we found that all sequences for both regions form a single monophyletic cluster, while on the other hand subtype B sequences clustered at different points in the tree. For subtype B, we found either non-clustered sequences or small regional clusters. In some cases clusters consisted only of sequences from a single region. Phylodynamic analysis of subtype F suggested that the time of the most recent common ancestor (tMRCA) for subtype F1 in Galicia was in 2006 (median estimate, 95%HPD: 2004-2008).

Conclusions: Subtype F circulates at high prevalence in two different areas of the Northwest of Spain, suggesting that HIV-1 epidemic in this region has distinct characteristics to the rest of Spain. Notably subtype F has been spreading among MSM as the result of single point introduction, suggesting local spread. In contrast to F, subtype B has been introduced from multiple sources in these regions. Phylodynamic analysis showed that subtype F epidemic is recent initiated during the last years.

No conflict of interest

Abstract: P_9

Viral Evolution & Genetic Diversity

Insertions and deletions in p6-gag correlate with the predicted cellular tropism of HIV-1 V3 sequences

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Background: Highly active antiretroviral therapy consists of six different drug classes and leads to sustained suppression of viral replication in the majority of patients. However compliance problems facilitate the emergence of drug resistant viruses even jeopardizing the effectiveness of these newer highly potent treatment regimens. One drug used in these modern treatment regimens is maraviroc blocking the cellular coreceptor CCR5. The treatment failure of maraviroc has been

associated with a viral switch of the coreceptor usage. Since the switch of the coreceptor usage is also associated with a switch of the cellular tropism of HIV, multiple adaptations throughout the viral genome are most likely needed due to specific restriction factors and differences of the cellular machinery in infected cells. Therefore, we asked whether HIV isolates using either CCR5 or CXCR4 coreceptors carried specific mutational patterns in the C-terminal Gag, protease or reverse transcriptase.

Material and Methods: HIV-1 subtype B isolates (n=267) obtained for routine diagnostics from patients before the start of antiretroviral treatment (n=94) and after failure of antiretroviral treatment with (n=91) and without (n=82) primary resistance mutations were analyzed in the C-terminal Gag region, protease, reverse transcriptase and the V3 loop. HIV-1 tropism was determined using geno2pheno(coreceptor) with false positive rates <5, 5-15,>15 indicating X4-capable, intermediate, R5viruses, respectively.

Results: The genotypically classified X4-capable and R5 HIV-1 isolates differed in the Gag protein. Insertions of two or more amino acids in p6-gag between positions 470 to 485 were more frequent in R5-tropic viruses and deletions in p6-gag were accumulating in X4-capable viruses, specifically deletions between aa 472 and 479 ($p<0.005$). Not surprisingly X4-capable viruses had significantly more often resistance associated PR ($p<0.005$), RT ($p<0.01$) and Gag ($p<0.001$) mutations. But even in the subgroup of treatment-experienced viruses with primary resistance mutations X4-capable viruses tend to have more resistance-associated PR mutations (R5-tropic: 2.1(+/-2.3) vs. X4-capable: 2.7(+/-2.2) and significantly more often resistance-associated Gag mutations ($p<0.005$), whereas RT mutations were equally distributed. Specific mutations in the V3 loop of R5-tropic viruses have been found to influence the cellular tropism of HIV either macrophage-tropic or T-cell tropic. If the R5 viruses were grouped accordingly, 40 HIV-1 isolates would be classified as macrophage-tropic. These viruses tended to have more treatment associated Gag mutations than the other R5 viruses and harbored more often PR mutation I54V ($p<0.05$) as well as depletions in p6-gag ($p<0.05$), especially between aa 480-483.

Conclusions: The cellular tropism is especially depended on the Gp120 protein of HIV-1 characterizing the coreceptor usage (R5 and X4-capable) and its binding affinities to CD4 and CCR5 receptors (T-cell- and macrophage-tropic) but also other HIV-1 proteins seem to facilitate viral replication in different sets of cells. The correlation between the cellular tropism of HIV-1 and Gag insertions and deletions points to complex cell specific interactions of viral and host factors.

No conflict of interest

Abstract: P_10

Viral Evolution & Genetic Diversity

HIV-1 dissemination in Cyprus, the Eastern European frontier: Phylogeographic and temporal dynamic analyses of viral sequences nearly a decade

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Background: This study aimed at describing the genetic subtype distribution of HIV-1 strains isolated in Cyprus and their epidemiological link with HIV-1 strains from other countries. Previous studies showed a very heterogeneous genetic pool of HIV-1 strains in Cyprus and this directed us to determine the dynamics of the local HIV-1 infection during 2005-2012 by characterizing HIV-1 strains isolated from 334 HIV-1 infected individuals.

Materials & Methods: Nucleotide sequences of the pol (protease and reverse transcriptase) were amplified by RT-nested PCR using diluted plasma HIV-1 RNA. Drug resistance was determined according to the Genotypic Resistance Interpretation Algorithm guidelines of the Stanford HIV Drug Resistance Database. Drug resistance associated mutations were classified using the WHO list for surveillance of transmitted drug resistance. Subtypes were

based on the Rega subtyping tool. We analyzed the sequences for phylogenetically related clusters using the maximum likelihood method with the general time reversible model for nucleotide substitutions. A cluster was defined when the bootstrap value was 90% or higher and the p-distance < 0.03.

Results: A total of 334 patients were included, of whom 211 (63.2%) were antiretroviral naïve. The most common subtype was B (53.1%), followed by subtype A1 (19.4%) and subtype C (9.0%). Most patients originated from Western Europe (77.9% who were predominantly from Cyprus), followed by individuals from Eastern Europe and Central Asia (EECA - 9.3%) and sub-Saharan Africa (9.0%). Patient characteristic varied by geographical origin with individuals from Western-Europe being more frequently male (87.4%, 35.5% and 43.3% for respectively, Western-Europe, EECA and sub-Saharan Africa), infected through MSM (59.1%, 9.7%, 10%) and treatment experienced (41%, 22.6%, 20%). Transmission of drug resistant HIV was uncommon with a prevalence of 3.3%. The prevalence of acquired resistance was higher (26.8%). The most common mutations were K103N (0.9% naïve, 12.2% experienced), M184V (0.5% vs 9.8%) and M46L (0.9% vs 0.5%). We identified a total of 51 clusters including 158 patients (47.3%). A number of 112 antiretroviral naïve patients were part of a cluster (53.1% of all treatment naïve patients). A proportion of 43.1% of clusters included at least three patients. The largest cluster consisted of 12 patients. A number of 14 clusters consisted of both treatment-naïve and treatment-experienced patients. Patients that were not part of a cluster were more likely to originate from Sub-Saharan Africa (3.2% not in a cluster, 14.2% part of a cluster) ($p<0.001$). Conversely, patients from Central Asia and Eastern Europe were more likely to be part of a cluster (12.7% of patients in a cluster and 6.3% of patients not in a cluster) ($p=0.06$).

Conclusions: The polyphyletic nature of HIV-1 infection in Cyprus is reconfirmed and the results of the study underline the impact of population movements in HIV-1 transmission and dissemination in the island. The transmissions of HIV-1 drug resistant strains are remarkably lower than other European countries.

No conflict of interest

Abstract: P_11

Viral Evolution & Genetic Diversity

Continued increase of the prevalence of HIV-1 non-B subtypes: Update from the German HIV 1 Seroconverter Study

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Background: Currently, nine genetically distinct subtypes (A-D, F-H; JK)) and 61 circulating recombinant forms (CRFs) are defined which are divergently spread world-wide. Although subtype B is the most common subtype in Europe and predominates HIV-1 infections in men who have sex with men (MSM), an increase of non-B subtypes was observed in several European countries. Studies revealed an impact of some HIV-1 subtypes and CRFs on disease progression. Potential differences on treatment response and preferential transmission routes of HIV subtypes are also discussed. We evaluated the dynamics of spread of HIV-1 subtypes in patients with a known date of infection (German HIV-1 Seroconverter Cohort).

Methods: From 2060 drug-naïve HIV-1 patients with a documented seroconversion between 1996 and 2012 the HIV-1 *pol* subtype was determined. Men who have sex with men (MSM) represent the major transmission group of the study population (87.8%). The study population is representative for MSM in Germany with respect to reported newly diagnosed patients. HIV-1 *pol*-sequences (99 amino acids of the protease and 296 amino acids of the reverse transcriptase) were determined from PCR-amplified viral RNA (ViroSeqTM HIV-1 Genotyping System, Abbott). Subtype was determined using the REGA-tool (version 2.0) and by additional

phylogenetic analysis with an extended panel of reference sequences (Neighbor joining and Maximum Likelihood; PHYLIP package version 6.5, Felsenstein J). Bootstrap analysis was performed with 1000 pseudo data sets. The χ2 test was applied to compare categorical variables. Logistic regression was used to calculate time trends.

Results: In the study cohort 91.2% (n= 1879/2060; CI 95% 89.9 - 92.4) of the patients were infected with HIV-1 subtype B strains. Non-B infections were identified in 8.8% (n=181; CI 95% 7,6 - 10.1) of the study patients: The predominant non-B subtype was the recombinant CRF02_AG (26%), followed by subtype C (20%), subtype A (19%), CRF01_AE (14%) and 13% unclassified unique recombinant forms (URFs). HIV-1 non-B infections were observed in all transmission groups with different prevalence. Non-B strains were most frequently identified in HIV patients infected by heterosexual contacts (56.7%), 29.2% non-B infection occurred in IDU and 3.4% of MSM were infected with non-B viruses. The mean prevalence per calendar year of non-B infections increased significantly over time (1996-2012; p<0.001). Despite the low overall non-B prevalence in MSM, non-B infections were identified for the first time in 2001 and the prevalence increased since then (p<0.001).

Conclusions: Preferential transmission routes of specific non-B subtypes could not be identified. Despite the higher prevalence of non-B strains in heterosexually transmitted infections an increase of non-B strains in MSM was observed. The rise of HIV-1 non-B infections in MSM should be further monitored to follow the dynamics and to analyze its impact and consequences.

No conflict of interest

Abstract: P_12

Viral Evolution & Genetic Diversity

Incomplete Apobec3G suppression by mutations in Vif promotes evolution from CCR5 to CXCR4 tropism

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Background: Some Vif mutations favor incomplete Apobec3G (A3G) suppression. In this setting, the increased burden of G-to-A mutations introduced by A3G can contribute to HIV genetic diversity and to HIV escape from antiretroviral drugs. We have recently shown an increased rate of G-to-A mutations in the CXCR4-using NL4.3 with suboptimal Vif activity in a cell line model. Here, we investigate whether the presence of specific Vif mutations can contribute to the generation of G-to-A mutations at V3 positions critical for CXCR4 usage in primary cells infected by a CCR5-using virus.

Methods: The CCR5-using 81A-Vif_{wt} and the 81A-Vif_{K22E}, 81A-Vif_{E45G} mutants with suboptimal activity against A3G were used to infect PBMCs. PBMCs were co-cultured with fresh PBMCS obtained by different donors every 7 days for a total of 21 days (experiments performed in triplicate). Frequency of G-to-A V3 mutations recovered from proviral DNA was analyzed by ultradeep sequencing (UDPS) at 7, 14, 21 days post infection (dpi). After shorah correction, mutations detected in both forward and reverse primers in >4 reads were considered. The False Positive Rate [FPR] by Geno2pheno was used to infer HIV tropism. To assess the correlation between Vif mutations and co-receptor usage, 769 paired HIV B

subtype Vif and V3 RNA sequences from Los Alamos DB were analyzed.

Results: In PBMC, 81A-Vif$_{K22E}$ and 81A-Vif$_{E45G}$ show a decreased p24 production compared to p81A-Vif$_{wt}$ (mean log reduction=3.1 for K22E and 1.2 for E45G).
By UDPS, no G-to-A mutations are detected in 81A-Vif$_{wt}$-infected PBMC at each time point analyzed (0/14,642 reads at 7dpi, 0/22,084 at 14dpi, 0/23,013 at 21dpi). G-to-A mutations at 2 V3 positions are observed in 81A-Vif$_{E45G}$-infected PBMC (prevalence from 0.03% [5/22,448] to 0.06% [8/22,448]). Conversely, a progressive enrichment of G-to-A mutations is detected in 81A-Vif$_{K22E}$ infected PBMC (2.2% [363/17,160] reads at 7dpi, 2.0% [220/10,672] at 14dpi, 3.0% [313/10,359] at 21dpi). G-to-A mutations account for $\geq$98% of the overall genetic variability, and occur at 6 V3 positions with 6/6 resulting non-synonymous (R9K, G15R, G24E, E25K, G28E/R, D29N). The presence of G24E and E25K strongly decreases the FPR (from 24.7 for wt to 6.8 for G24E and 5.0 for E25K), indicating CXCR4 usage acquisition. Consistent with this result, the introduction of G24E or E25K in B-subtype gp120 strongly increases CXCR4 N-terminus binding affinity for V3 (-40.1Kcal/mol for wt versus -510Kcal/mol for G24E and -522Kcal/mol for E25K). G15R resides in V3 crown proposed as vaccine target.
Finally, the analysis of paired V3 and Vif RNA sequences shows that the presence of at least one mutation in Vif$_{22}$ and/or Vif$_{45}$ correlates with increased prevalence of CXCR4 usage (44.8% versus 31.5% in Vif$_{wt}$ P=0.031).

Conclusions: A3G incomplete neutralization by single Vif mutations favors the generation of a proviral reservoir with an increased capability to use CXCR4 and to potentially escape neutralizing antibodies. This has implications for the success of CCR5 antagonist-based dual therapy and for immunological HIV control.

No conflict of interest

Abstract: P_13

Viral Evolution & Genetic Diversity

Peak of acute HIV and HCV infections following prominent MSM festivals in Berlin

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Introduction: During summer time prominent street festivals like Christopher street day (CSD) or similar events are under suspicion to contribute to HIV transmission rates all over the world. However, data contributing or refuting such suspicions are rare. Here, we analyzed frequencies of acute and newly diagnosed HIV, HCV and syphilis infections and their monthly distributions in Berlin for the years 2011-2013.

Methods: All samples were tested during diagnostic routine and analyzed retrospectively. HIV and HCV acute infections and new diagnoses were monitored serologically and identified according ELISA and immunoblot criteria. If available, viremia in the same sample or another within less than six weeks and serological follow-up samples were used to confirm diagnosis during acute infections. Syphilis infections were diagnosed by serologic screening and confirmation testings from years 2011-13 were screened. Finally, all newly identified infections were counted in each month between January 2011 and December 2013.

Results: Overall more than 100000 samples were analyzed in 2011-2013. In 2011 8.3% (n=8), in 2012 13% (n=11), and in 2013 24% (n=20) of all newly diagnosed HIV infections were acute infections without fully developed immunoblot. The mean number of new HIV diagnoses per month was constant (9;8;8) over the years, and a slight increase to 10 to 15 new infections could be seen in June and July in all three years representing the maxima in 2012 and 2013 and the second highest value in 2011. A very similar pattern was observed for HCV with maximal numbers of new diagnoses in June each year (n=28;28;25), while syphilis diagnoses turned out to have maxima two to

four months later (35 in August 2011, 25 in October 2012, and 46 in October 2013). In 2013, an unusual high percentage of acute HIV infections in July could be observed (46%, 6 of 13), which was associated to severe course of primary infection in some patients. Sequence analyses showed the same virus in 2 of 4 viral sequences derived from patients with severely symptomatic acute HIV infection. So far, we could not identify any viral property or particular mutational pattern of these strains.

Conclusions: We report a peak of recent and recently diagnosed HIV, HCV and syphilis infections following MSM associated major festivals in Berlin during summer time in all three years. Although the absolute numbers of acute infections each month were low, the parallel increase of new HCV infections in the same time seems to confirm the association to the summer happenings. The longer time for syphilis diagnoses may be explained by the longer time until syphilis may need to cause apparent symptoms. The finding of an HIV strain infecting two patients with severe primary infection is interesting and needs further investigation. In summary, we found that indeed summer festivals frequently visited by MSM may contribute to transmission rates of sexual transmitted diseases like HCV, HIV and syphilis substantially in Berlin, and that these findings should influence prevention policies in Germany and if indicated also in other countries.

No conflict of interest

Abstract: P_14

Viral Evolution & Genetic Diversity

Multiple HCV Reinfections in HIV-positive Patients in Germany

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Introduction: Due to the routes of transmission co-infection of HIV-1 and HCV is common. With increasing hepatitis C treatment options for a cure, it became obvious that re-infections can occur. Furthermore, a rising incidence of sexually transmitted HCV re-infections in HIV-infected men who have sex with men (MSM) has been reported. Here, we analyze the frequency of HCV re-infection in HIV-infected MSM displaying episodes of acute HCV infection in Germany.

Methods: In total, 402 HIV-infected patients were screened for acute HCV infection, and diagnosed between January 2001 and May 2013 at four major German HIV and hepatitis care centers (Berlin, Bonn, Frankfurt, and Hamburg). Analyzed in more detail were patients with more than one sexually acquired HCV infection. Re-infection was defined by detectable hepatitis C viremia either after sustained virological response (SVR) or after spontaneous clearance (SC), and genotype switch or confirmed by phylogenetic analysis.

Results: 48 HIV positive MSM were identified with HCV re-infection, among them eleven with a third episode and one patient with four episodes. At the first episode, 43 and 5 patients had a SVR or SC, respectively. The second episode was accompanied by a genotype switch in 29 (60%) patients. The other 19 patients with the same genotype than in episode 1 were identified to be re-infected by genetic distance similar than in independently and unrelated infected patients of the cohort, or by the length of aviremic time period. All patients with a period of less than 18 months between both diagnoses showed a genotype switch, except for one patient that was treated for 24 weeks but had a documented SVR 24 weeks thereafter. 30 and 9 patients showed again SVR or SC. Eight patients developed chronic hepatitis. One was lost to follow-up. In this episode, neither HCV genotype switch nor IL28B genotype were associated with spontaneous clearance. However, SC rates at second episode were significantly higher for patients with SC at first episode (3 of 5, 60% versus 6 of 43, 14%, p=0.03). Of note, two of five patients with SC at the first episode were re-infected with the same genotype.

Conclusions: In the high-risk group of HIV positive MSM-patients in Germany multiple HCV re-infections do occur, with or without

genotype switch, and with prior spontaneous clearance of previous episodes. In this large case series, except for spontaneous clearance at the first episode, no factor was of value in clinical decision-making for early therapeutic intervention of acute HCV re-infection. The identification of re-infections in patients with the same genotype and within a short time period is hardly to discriminate from relapse. This may need ongoing investigation also in view of upcoming shorter HCV treatment periods in the near future to identify epidemiological aspects as we observed in Germany rather than assuming therapy failure while re-occurrence of hepatitis C viremia after therapy has been stopped.

No conflict of interest

Abstract: P_15

Spread of Drug Resistance

Changes in HIV-1 baseline drug-resistance in Spain from 2007 to 2013

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Background: We aim to characterize transmission of drug resistance (TDR) mutations and non-B subtypes in CoRIS, a cohort with wide territorial representation in Spain, from 2007 to June 2013. In addition we have evaluated clinical resistance to first line reverse transcriptase and protease inhibitors, and predicted the efficacy of preferred first line regimens.

Methods: CoRIS is a multicenter cohort of adult HIV naïve patients. By 2013, 27 sites from the 34 Spanish centers participating in the cohort contributed with 3479 patients to the study. TDR associated mutations were evaluated following the WHO 2009 updateHIV Subtype and PDR to first line drugs was investigated using Stanford HIV Db algorithm v 6.3.1. Sex, age, transmission category, educational level and country of origin, CD4 count and Viral Load (VL), CDC stage, and delayed diagnosis (CD4 count <350 cells/mm^3 at diagnosis) were recorded.

Results: Throughout the study period, TDR prevalence was 7.0% (6.1-7.8). Similar prevalence was found for NRTIs 3.6% (2.9-4.2) and NNRTIs 3.2% (2.6-3.8). Resistance to PIs was 1.1% (0.8-1.5). TDR prevalence decreased from 7.6% in 2007 to 4.6% in 2013. TDR to more than one family was uncommon (0.8%; 0.5-1.1). 574 subjects had non-B subtypes [16.5% (15.3-17.7)]. Non-Bs increased over time [12.2% (9.2-15.2), 2007 to 21.6% (15.6-27.7) in 2013 (p trend=0.015). CRFs accounted for 53.8% (n=309) of non-Bs (8.9% prevalence). Using Stanford Algorithm, clinical resistance to first line ARVs was 10.5% (9.5-11.5), with a decrease from 10.3% in 2007 to 5.7% in 2013. Clinical resistance was the highest for first line NNRTIs (5.1% for Rilpivirine, 4.7% for Nevirapine and 4.2% for Efavirenz). For first line NRTIs (Tenofovir, Abacavir, 3TC and FTC) clinical resistance ranged from 0.7% to 2.1%. The lowest clinical resistance was found for first line PIs (Atazanavir, Darunavir and Lopinavir) ranging from 0.1 to 0.7%. First line PI based regimens have shown the highest predicted efficacy (2.3% to 2.6% for not having a fully active regimen, p<0,001 compared to all NNRTI based regimens (5.8% to 6.8%). Interestingly, if mutation E138A is not considered relevant for resistance, first line Rilpivirine based regimens show a significantly (p<0.001) lower predicted efficacy than the first generation NNRTI based ones, and resistance to Rilpivirine falls down to 1.8%.

Conclusions: Transmitted Drug Resistance (TDR) in Spain remains low and with no trend over time during the study period. Non-B subtype infections are on the rise with a majority of CRFs among the non-Bs. Although clinical resistance shows a decrease from 2007 to 2013, baseline resistance to NNRTIs remains a problem of concern for the selection of first line regimens.

No conflict of interest

Abstract: P_16

Spread of Drug Resistance

Predictors of HIV-1 transmitted drug resistance and transmission networks in the Leuven cohort

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Background: Surveillance of transmitted drug resistance (TDR) and characterization of HIV-1 transmission networks in local epidemics are important to understand the dynamics of new infections and its impact on antiretroviral therapy (ART). We aimed to determine the prevalence of TDR, factors associated with TDR, and the contribution of transmission clusters to the spread of TDR.

Materials & Methods: A total of 778 patients enrolled between 1998 to 2012 at the AIDS Reference Center in Leuven (Belgium) were included. HIV-1 *pol* sequences obtained for drug resistance testing (DRT) were used to estimate the prevalence and clinical impact of TDR using the WHO-2009 surveillance list and REGAv9.1, respectively. Sequences were subtyped using REGAv3, COMETv2 and manual phylogenetic analysis. Four additional control datasets were included to evaluate TDR transmission clusters with maximum likelihood and Bayesian techniques. Logistic regression and Bayesian network analyses were used to evaluate factors associated with TDR and transmission clusters.

Results: 58.4% of patients originated from Belgium, 42.8% reported men who have sex with men (MSM) as risk of transmission, 86.5% had a chronic infection and 52.2% were infected with subtype B. The overall TDR prevalence was 9.6% (95% confidence interval (CI): 7.7-11.9), mainly related to singletons (70.7%). Resistance for nucleoside reverse transcriptase inhibitors (NRTI) was 6.5% (CI: 5.0-8.5), for non-NRTI it was 2.2% (CI: 1.4-3.5), and for protease inhibitors 2.2% (CI: 1.4-3.5). A stable overall TDR and a significant parabolic trend (peak in 2008) of NNRTI-TDR were found (p=0.019). Factors in univariate analysis significantly associated with TDR were male gender, Belgian origin, MSM, recent infection, part of a transmission cluster and infection with subtype B, while only the latter factor remained in the multivariate and Bayesian network analyses. Amongst the patients with viruses carrying TDR, 83.3% who started ART achieved a sustained undetectable viral load, whereas half would likely have received a suboptimal ART if the DRT had not been performed.
114 transmission clusters were identified, of which 16 included 41% of the TDR patients. Subtype B clusters were predominant (eight clusters and five pairs), followed by one CRF02_AG cluster and one pair for subtype C and CRF01_AE. Belgian origin, MSM risk factor and infection with subtype B were associated with TDR clusters in the univariate analysis. Although none of these factors were significant in the multivariate analysis, Bayesian analyses showed a dependency between subtype B and transmission clusters. When excluding the subtype variable to evaluate sequence-independent factors associated with TDR or transmission clusters, only dependencies with low support were found: male gender with TDR,

Belgian origin and MSM risk factor with transmission clusters.

Conclusions: TDR prevalence was stable and similar to other European reports. However, an up-and-down trend for NNRTI was found in this local epidemic. TDR and the spread of resistance was mainly related with subtype B infected individuals, albeit no specific subpopulation could be significantly identified for targeting prevention policies. The high number of chronically infected drug naive individuals and their involvement in TDR transmission clusters highlights the need to improve early diagnoses and continuous monitoring.

No conflict of interest

Abstract: P_17

Spread of Drug Resistance

Primary resistance to second-generation Non-Nucleoside RT inhibitors in HIV-1 recent seroconverters in Spain

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Background: Monitoring transmission of drug resistance mutations provides valuable information for maximizing antiretroviral treatment success in newly infected persons. Second-generation NNRTIs were available as first-line antiretroviral therapy in Spain since year 2009. Herein, we report the prevalence of transmitted drug resistance mutations to NNRTIs in the Spanish cohort of HIV-1 seroconverters.

Methods: Retrospective, cross-sectional analysis performed in the national database of recent HIV-1 seroconverters (confirmed <12 months from exposure). Demographics and laboratory parameters (plasma HIV-RNA, CD4 counts, HIV-1 subtype, tropism and major DRM) were recorded from January 1997 to December 2012. All mutations associated with resistance to NVP, EFV, ETR and RPV were considered.

Results: From a total of 1030 recent seroconverters included in the cohort, 408 were diagnosed after January 2009. We will focus all subsequent analyses in this group (males 98.8%; median age 31 years; MSM 98.5%). Median estimated time from infection was 8 [IQR: 5-12] months. By the time of diagnosis, median plasma HIV-RNA and CD4 counts were 4.5 [3.9-4.9] log copies/mL and 549 [415-741] cells/mm^3, respectively. Overall, 16.4% carried HIV-1 non-B subtypes and 21.1% were infected with X4 viruses.
Following the release of the last updated list of transmitted drug resistance mutations (Bennett et al, 2009), the overall rate was 4.7% for NNRTI in our cohort. However, it increased up to 13.3% ($p<0.0001$) when all mutations currently associated with NNRTI resistance (Stanford rules, September 2013) were considered. The prevalence of any mutation change was 13.3% for NVP, 8.6% for EFV, 9.8% for RPV and 9.2% for ETR. Changes recorded were as follows: A98G (0.2%), K103N (2.2%), E138A (4.3%), E138K (1.4%), V179D/E (2.6%), Y188H (0.2%), G190A (0.2%), and H221Y (0.2%). Predicted significant impaired response (intermediate or high level resistance using Stanford rules) was 1.7% for RPV and absent for ETR.

Conclusions: The rate of transmitted resistance mutations to second-generation NNRTIs in HIV-1 recent seroconverters is around 10% in Spain. It is largely driven by changes at position E138. Mutation lists should regularly be updated in order to accurately estimate the rate of drug resistance transmission in newly HIV-1 infected individuals.

No conflict of interest

Abstract: P_18

Spread of Drug Resistance

Pronounced potential of resistance mutations to persist in transmitted drug resistant HIV-1 strains

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Background: Generally, it is assumed that resistance mutations in the HIV-1 genome are rapidly lost during drug-naïve course of infection. In Germany, transmitted HIV resistance (TDR) to antiretroviral drugs (protease inhibitors (PI) and reverse transcriptase (RT) inhibitors (NRTI, NNRTI)) is monitored since 1997 in an open long-term observational HIV-1 seroconverter cohort. Treatment –naïve base-line samples and about yearly follow-up samples and data are collected from the study patients with a documented date of seroconversion. The aim of the study was to analyse the potential of the most prevalent TDR mutations to persist in vivo in transmitted drug resistant strains.

Materials & Methods: 2060 *pol*-sequences from 2128 base-line samples of drug-naïve seroconverters (year of seroconversion 1996-2012) and from 1794/2095 follow-up samples were determined (ViroSeq™ HIV-1 Genotyping System, Abbott or in house method). HIV-1 resistance mutations in the *pol*-sequences (amino acids 1-99 amino acids of protease and 1-296 of RT) were identified according to the surveillance drug mutation (SDRM) list for drug-naïve patients. Prevalence of transmitted drug resistance, resistance classes and resistance mutations, and time trends of the yearly mean prevalence were calculated. Follow-up samples of patients infected with HIV carrying the most prevalent SDRM were analysed for persistence of resistance mutations. Categorical variables were compared by $\chi 2$-test or Fisher's exact test. Time trends were calculated by logistic regression. Kaplan-Meier survival analysis (KM) was performed to determine the median/mean KM estimates (right-censored) of duration of persistence of the most prevalent resistance mutations.

Results: The mean prevalence of transmitted drug resistance (TDR) was 12.0 % (250/2060; CI 95% 10.7-13.5) showing no significant decrease between 1996 and 2012 (p=0.118) in the study population. NRTI-resistance predominated with a mean of 6.1%, followed by 2.4% NNRTI- and 2.0% PI-resistance. Prevalence of NRTI-resistance decreased over time (p=0.016) whereas prevalence of NNRTI- and PI-resistance remained stable. The most prevalent resistance mutations were thymidine-analogue resistance mutations (M41L, D67N, T215CDEISV, K219EQR), NNRTI mutation K103N and PI resistance mutations M46L and L90M (0.7-3.7% prevalence). Duration of observation varied between 18-33 person months. The range of mean KM estimates for the persistence of these mutations was 5.1-7.8 years (CI 95% 3.6-10.9). In particular, the K103N mutation persisted with a mean KM estimate of 7.8 years (CI 95% 4.7-10.9).

Conclusions: The decrease of transmitted NRTI resistance seems to reflect the decrease of treatment failure described in various observational studies. The pronounced potential of some resistance mutations to persist for years in transmitted HIV-1 might be related to their known low impact on viral replication competence (e.g. K103N, T215 revertants). In vivo persistence of resistance mutations as observed in this study is likely to contribute to the continued spread of resistant HIV in newly infected patients. Onward transmission of primary resistant HIV therefore adds probably to the stable level of TDR observed in the newly infected population.

Part of the data were presented at the 12th European AIDS conference, Brussels, Belgium, Oct 16-19, 2013

No conflict of interest

Abstract: P_19

Spread of Drug Resistance

Trend of HBV genotype and RT/POL mutations prevalence in a cohort of chronic infected patients, Genoa, Italy

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Background: Lamivudine-LAM was the first nucleos(t)ide analogue-NUC approved for CHB (1998), followed by Adefovir-ADV (2002), Entecavir-ETV (2005), Telbivudine-TBV (2006), Tenofovir-TDF (2008) and Emtricitabine-FTC, not currently approved for treatment of CHB, but commonly used in combination treatment in coinfected HIV-HBV patients. In the last years TDF and ETV have been the most widely administered drugs, due to their potency in HBV inhibition and high barrier to resistance, however, until a few years ago, LAM was the most widely used drug in our country.
The aim of this study was to determine how mutations conferring HBV resistance to NUC and HBV genotypes have evolved over the last 9 years under pharmacological pressure.

Material & Methods: In the Hygiene Unit of the IRCCS AOU San Martino-IST, Genoa, Italy, a retrospective survey was performed on genotypic resistance tests in samples obtained by 436 samples out of 353 routinely examined patients from January 2005 to December 2013. Sequences were obtained by Trugene® HBV Genotyping kit (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). The following: A181T/V, A194T, M204V/I/S, I233V, N236T and M250V primary mutations and V173L, L180M, T184A and S202G secondary mutations were evaluated.

Results: Every year from 2005 to 2013, respectively, 25, 18, 39, 51, 58, 49, 70, 64 and 62 sequences were analyzed, for a total of 436. With regard to genotype prevalence, through the years the rate of D genotype declined from 95% to 67.7%, whilst among the others genotypes, a spread of B, C and especially A genotype was observed. The last one showed an increase from 4% to 22.6%.
As to mutation prevalence, 139/436 (31.9%) analyzed sequences revealed the presence of at least one mutation conferring drug resistance. The most prevalent mutation was M204V/I, followed by L180M, which passed from 61% to 36% and from 39% to 32% through the years, respectively. LAM resistance declined from 44% to 16%; ADV and ETV resistance was seen in 2006 and in 2008 at a stable rate (5.6% and 6.4%, 3.9% and 4.8%, respectively). In one LAM resistant patient, in 2011 also the A194S mutation was detected, able to confer TDF resistance. During the period of observation the prevalence of wild type sequences increased from 56% to 81%.

Conclusions: This study showed that D genotype was overall the most represented, but over the years a progressive decrease of the D genotype prevalence and a gradual increase of non-D genotypes were observed. In fact, a rise of the spread of A genotype could be monitored, followed by B and C, probably due to the immigration.
A progressive decrease in prevalence of mutation conferring LAM resistance was shown. This is probably due to the progressive substitution of this drug, in the clinical practice, with drugs with a higher genetic barrier and strong antiviral activity. Expectedly, further resistance was also found to ETV and ADV. With regard to ETV, resistance was mainly observed in LAM experienced patients. Only one patient had a virus harboring the A194S mutation, able to confer TDF resistance.

No conflict of interest

Abstract: P_20

Spread of Drug Resistance

Transmission clusters of drug resistance in subtype B in Portugal

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Background: Antiretroviral drug resistance can be a problem for first line therapy decision, and it can be found in 8.4% of the drug naive HIV infected patients in Europe. The main goal of this study was to develop a phylogenetic framework to detect clusters of transmitted drug resistance (TDR) in the Portuguese population of HIV-1 infected patients and to discriminate between transmission of drug resistance from patients failing treatment or from other drug naive (DN) patients.

Materials & Methods: The Portuguese HIV drug resistance database contained *pol* sequences from 8979 patients followed in 22 hospitals from the South of Portugal between January 2001 and April 2013. We estimated a coverage of 60% for treated and 30% for newly diagnosed drug naive patients in this region. Subtyping was done using REGAv3 and COMET v0.5. 4538 subtype B sequences from 3561 patients (1687 sequences from DNs+2851 from treated) were used to build Maximum likelihood trees (RAxML, evolutionary model GTR+Γ+I, 1000 bootstrap replicates). Transmission chains were identified using variable thresholds of bootstrap support (70 to 90%) and pairwise distance (0.015 to 0.004), thus allowing to incorporate for uncertainty in the definition of transmission clusters. TDR was defined using the WHO-2009 list. Python scripts were used to traverse the tree and identify clusters with TDR.

Results: TDR prevalence was 11.3% (95% CI: 9.8%; 12.9%). TDR associated with PI resistance was 3.9% (3.0%; 4.9%), NRTI TDR was 5.8% (4.8%; 7.1%) and NNRTI TDR was 4.7% (3.8%; 5.9%). We mainly found single class TDR (8.5%, (7.2%; 9.9%)). The number of transmission clusters varied between 375 and 496, depending on the thresholds considered, 43-44% of these clusters contained only DNs. Between 79 and 113 of transmission clusters had at least two patients with TDR. Of these clusters with TDR, those with two TDR patients were the most common (11.4-13.2%), followed by three TDR patients (3.7-6.0%), four TDR patients (2.9-3.1%) and five or more TDR patients (2.7-3.4%). We had significantly more clusters with NNRTI TDR than for the other drug classes (14.1-18.3% vs, 4.5-6% for PI TDR and 6.1-8.2% for NRTI TDR, p-value<0.01). The proportion of clusters with TDR containing only DNs was largest for NRTI TDR clusters (44.1-50%), followed by NNRTI TDR clusters (40-43.4%) and PI TDR clusters (32-35%). Similarly, the proportion of clusters with TDR with only DNs was largest for single class resistance (45.4-47.0% vs 28.6-36.4% for double class resistance and 20-25% for triple class resistance).

Conclusions: We show that this phylogenetic framework could represent a valuable tool to characterize transmission clusters of TDR. In Portugal a substantial number of transmission clusters contain at least two patients with TDR, and these are more common for NNRTI TDR than for other drug classes. However, transmission clusters involving only DN patients occurred more frequently in clusters involving NRTI TDR than for other drug classes.

No conflict of interest

Abstract: P_21

Therapeutic Challenges in Resource-limited settings

Predictors of attrition and immunologic failure in HIV-1 patients on self-paid HAART in Mozambique

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Background: Mozambique suffers from one of the highest HIV/AIDS burdens in the world, with HIV-1 prevalence of 21% in the southern regions where the capital Maputo is based. In Mozambique, patients with medium-high socioeconomic status can afford to pay for laboratory testing and for their medication, thus having access to antiretrovirals different from those being currently recommended to the general population. The evaluation of retention in care and ART programmes in this country is limited. This is the first assessment of attrition and HAART effectiveness in the pay-for-care setting in this country.

Patients & Methods: Retrospective survey of antiretroviral-naïve HIV-1-infected patients attending 16 healthcare centres (HCC), one centrally located clinic (HCC1) and 15 small private clinics located around Maputo, which were grouped for convenience of analysis and named HCC2. The inclusion criteria were: residency in the Maputo province; ≥18 years of age at HAART initiation; documented date of start of HAART comprised of at least 3 antiretrovirals, available baseline CD4 T-cell count (for the six months before HAART initiation); follow-up of ≥3 months. Cox proportional hazard models were used to assess risk of attrition (no retention in care) and immunologic failure (defined according to the 2010 WHO recommendations).

Results: Overall, 142 patients who started HAART between 2002 and 2006 were followed-up for 22.2 months (12.1-46.7). Most patients (62, 43.7%) were from HCC1. The retention rate was 75%, 48% and 37% after one, two and three years, respectively. Median CD4 count, VL and age were similar between patients attending HCC1 and HCC2. Median baseline CD4 count was 185 cells/μL (126-290) and median baseline HIV-1 RNA was 5.3 (4.8-5.9) $\log_{10}$ copies/mL. Risk of attrition decreased at higher baseline CD4 count (AHR: 0.83; 95%CI: 0.70-0.97; P=0.022) and was related to the HCC of origin such that patients attending HCC2 had a higher risk than those attending HCC1 (AHR: 1.80; 95%CI: 1.16-2.79; P=0.013). The proportion of individuals with CD4 count ≤200 cells/μL was 55% (78/142) at baseline; it decreased to 6% (3/52) at 36 months. A biphasic kinetic of CD4 T-cell recovery was observed, characterized by a rapid average increase during the first semester followed by a slower increase thereafter. CD8 count showed small changes over time, while CD4/CD8 ratio increased to 0.5 (0.4-0.7) at 36 months (P<0.001). Among the patients with available HIV-1 RNA, 86% (64/74) achieved undetectable HIV-1 RNA levels. Resistance mutations to NRTIs and NNRTIs were observed in 3 patients with virologic failure. The rate of immunologic failure was 17.2% (95%CI: 12.6-22.9) per 100 person-years. Risk of failure was associated to higher baseline CD4 count (AHR: 1.30; 95%CI: 1.10-1.53; P=0.002) and decreased with baseline VL≥10,000 copies/mL (AHR: 0.33 [0.12-0.91]; P=0.033).

Conclusions: These results indicate that in resource-constrained countries HAART can be successful also when offered in different healthcare settings to populations self-paying HAART. Further studies are required to identify the causes for low retention rates in patients with low CD4 counts and to elucidate the association between healthcare setting and attrition in order to improve strategies to increase in-care retention levels.
No conflict of interest

Abstract: P_22

Therapeutic Challenges in Resource-limited settings

HCV antibody testing markedly overestimates the prevalence of HCV co-infection among HIV-positive patients in an urban sub-Saharan African setting

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Background: HCV seroprevalence rates in excess of 5% have been recently reported in some HIV cohorts in sub-Saharan Africa (SSA), suggesting an alarming disease burden for which there are no defined screening and management policies. The surveys have commonly employed EIA-based antibody assays for screening and rarely tested for HCV RNA to confirm a current infection. The purpose of this study was to determine the prevalence of HCV RNA positive infection in a large cohort of HIV-infected adults from Kumasi, Ghana, and relate the findings to anti-HCV antibody detection using three gold-standard assays.

Material & Methods: HCV RNA was detected in pools of 5 plasma or dry plasma spots (DPS) by real-time PCR, followed by testing of individual samples of positive pools. Assay sensitivity was 1000 IU/ml for pooled plasma and 5000 IU/ml for pooled DPS. Serum samples from 71 subjects (67 HCV RNA negative, 4 HCV RNA positive) were tested for anti-HCV antibody using the automated Architect platform (Abbott Diagnostics) and the plate assays ORTHO HCV 3.0 ELISA System with Enhanced SAVe (Ortho Clinical Diagnostics) and Monolisa HCV Ag-Ab ULTRA (BioRad). Samples that tested Architect reactive / HCV RNA negative were retested using the INNO-LIA HCV Score immunoblot assay.

Results: Overall, 9/1275 specimens tested HCV RNA positive, yielding a prevalence of HCV RNA positivity of 0.71% (95% CI 0.25-1.17%). Anti-HCV antibody reactivity was 9/71 (12.7%) by Architect, 9/71 (12.7%) by Ortho and 23/71 (32.3%) by Monolisa. Overall concordance rates were 53/71 (74.6%) for Architect and Monolisa, 65/71 (91.5%) for Architect and Ortho, and 53/71 (74.6%) for Ortho and Monolisa. The four HCV RNA positive subjects tested anti-HCV antibody reactive in all three assays with median (range) reactivity of 12 (7-13), 5 (4.9-5.3) and 6.4 (5.9-6.6) by Architect, Ortho and Monolisa respectively. Among 67 HCV RNA negative subjects, 5 (7.5%), 5 (7.5%) and 19 (28.3%) tested anti-HCV antibody reactive by the three assays respectively, with median (range) reactivity of 3.3 (1-5.2), 1.5 (1.1-4.5) and 2 (1-6). In this group, 4/67 (6%) samples were reactive in ≥2 assays including 2/67 (3%) that were reactive in all three assays. Of five Architect reactive/HCV RNA negative samples tested by INNO-LIA, one gave an indeterminate result (anti-C2 reactivity) and one gave a positive result (anti-C1, C2 and NS3 reactivity). Using samples from HCV RNA positive subjects (n=4) and samples testing Architect reactive/INNO-LIA positive or indeterminate (n=2) as reference of confirmed HCV infection, sensitivity and specificity were 100% and 95.4%, 83.3% and 93.8% and 83.3% and 75.4% for Architect, Ortho and Monolisa respectively.

Conclusions: Published HCV seroprevalence studies appear to overestimate the burden of HCV co-infection among HIV-positive adults in SSA. Careful consideration of surveillance and diagnostic methods is required to inform policy and practice. Increasing the Architect and Ortho assay cut-offs to 3.3 and 1.5 respectively may reduce the number of HCV RNA tests required. Larger studies are needed to confirm the validity of this approach.

No conflict of interest

Abstract: P_23

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Once daily single tablet of TDF/FTC/RPV in clinical practice: implication of antiretroviral drug resistance

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Background: Once daily single tablet of emtricitabine/rilpivirine/tenofovir disproxil fumarate (TDF/FTC/RPV) is effective and well-tolerated in antiretroviral treatment (ART)-naive HIV-infected patients with plasma viral load (VL) < 100,000 copies/mL. It has been recently approved by European Medicine Agency (EMA) for use as a switch in virologically-suppressed patients. We aimed to provide 'real-life' data on efficacy of TDF/FTC/RPV in treatment experienced-patients and the impact of resistance-conferring mutations to rilpivirine on virological success.

Methology: We performed a retrospective cohort study in the Rennes University Hospital. All patients who started TDF/FTC/RPV between September 2012 and May 2013 were enrolled. The primary endpoint was virological failure (HIV RNA > 40 copies/ml after 3 months, confirmed on a separate sample). Any data on resistance-associated mutations to RPV documented before patients enrolment were collected, and all patients with virological failure on TDF/FTC/RPV were tested for emergence of genotypic resistance (ANRS algorithm 2012, v22).

Results: We included 157 ART experienced patients (64 females, 93 males), with a mean age of 45 years ± 8.0. The mean number of different ART regimen before FTC/RPV/TDF introduction was 4 ± 2 and the mean CD4 cells count was 572 ± 231/mm^3. Before switching to TDF/FTC/RPV 141 (90%) patients had undetectable VL and 16 (10%) had detectable VL. Analysis of resistance-conferring mutation before FTC/RPV/TDF initiation was as follows: NNRTIs resistance–associated mutations, n=25/98 (including 11 with RPV resistance-associated mutation); NRTIs resistance-associated mutations, n=17/98. With a mean follow up of 7.8 ± 2.4 months, four patients (2.5%) experienced virological failure, three of them had detectable viral load when TDF/FTC/RPV was started (i.e. 168, 137, and 504,200 copies/mL as part of a salvage regimen for the latter). E138Q resistance mutation was present before starting RPV in one of these 4 patients, and 3 acquired *de novo* RPV-associated resistance mutation (E138K or E138A), including one who also acquired M184I mutation. No mutations to TDF or FTC were present in virologicaly failing patients at TDF/FTC/RPV initiation. 16 of the 17 patients presenting resistance-conferring mutation to NRTIs at baseline had undetectable VL at the end of follow-up. 10 of the 11 patients with RPV associated mutation (E138Q, E138K, E138A, K101E/K, Y181C, Y188L/H) at baseline had undetectable VL at the end of follow-up. Three patients (1.9%) discontinued RPV-based regimen because of adverse events (AEs): grade 2 nausea and grade 2 asthenia (n=2), and grade 3 rash (n=1). A total of 62 patients (39.5%) reported at least one AE, mostly of mild grade (78.9%). No serious AE was reported. The most frequent AEs were elevated transaminases (22.9%), asthenia (6.4%), insomnia and abnormal dreams (5.7%).

Conclusions: Despite a significant proportion of patients with RPV-associated mutation at baseline (11%), once daily single tablet TDF/FTC/RPV as switch regimen, was associated with low incidence of virological failure (2.5%) after 7.8 months follow up. The emergence of RPV-associated mutations occurred only in treatment experienced patients with detectable viral load.

No conflict of interest

Abstract: P_24

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Factors associated with atazanavir resistance mutations in HIV+ patients with virological failure

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Background: Previous studies have documented significant association between atazanavir (ATV) plasma concentrations or ATV genotypic inhibitory quotient (GIQ) and virological response in the overall HIV population. In the present study we specifically focused on HIV-infected patients with virological failure in presence of ATV resistance mutations.

Material & Methods: Steady state ATV plasma concentrations were measured in 157 HIV-infected patients referred to our Laboratory of Microbiology with a request of genotypic resistance test for virological failure. The genotypic resistance test was performed using the TRUGENE® HIV-1 Genotyping Assay (Siemens). ATV concentrations were assessed by a validated HPLC method (lower limit of quantification, LOQ 20 ng/mL). GIQ was calculated either as the ratio of Cthrough/number of ATV-associated mutations ratio, according to the International AIDS Society 2013 list, and as Cthrough/Stanford resistance mutation scoring ratio (version 6.3.0, S_GIQ). Multiple regression analysis were performed using ATV intermediated/high level resistance as the dependent variable and concomitant HAART (tenofovir vs. others), sex, ritonavir (rtv), ATV concentrations, ATV GIQ and the S_GIQ as independent covariates. Non-compliance was defined as ATV concentrations <20 ng/mL.

Results: Overall, 24.8% of the enrolled patients had intermediate/high level of resistance to ATV/r. Twenty-six percent of processed samples had ATV concentrations <20 ng/mL, a value that greatly differed from the percentage of samples below the LOQ observed in our general database that included data from more than 3000 assessments of therapeutic drug monitoring of antiretrovirals concentrations (around 4%). Non-compliance was found in 10.3% vs. 31.4% of patients with or without ATV resistance (chi-square test $p<0.001$).
By multivariate regression analyses we found that: a) non-compliance was significantly correlated with HIV viral load ($r = -0.173$, $p=0.039$); b) the only factor independently associated with ATV resistance was S_GIQ ($r = -0.201$, $p<0.001$), whereas no significant associations were found for concomitant HAART, ritonavir use, ATV concentrations or GIQ.

Conclusions: Our cohort of patients with a request of genotypic resistance test for virological failure while on ATV therapy had an unexpectedly high incidence of non-compliance compared with our general HIV population. Patients experiencing virological failure without ATV resistance mutations had lower compliance to ATV-base therapy compared with failing patients with ATV resistance mutations. S_GIQ was the most accurate predictor of ATV resistance compared with the unweighted GIQ and with ATV concentrations. No effect of concomitant HAART, tenofovir or rtv on ATV resistance selection was found.

No conflict of interest

Abstract: P_25

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Evaluation of virological efficacy and resistance profile in patients starting darunavir in clinical practice

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Background: So far, few data on the virological efficacy and resistance profile in patients starting darunavir are available outside clinical trials. For this reason, we analyzed a dataset from an Italian cohort of patients starting for the first time a regimen containing ritonavir-boosted darunavir (DRV/r) in clinical practice.

Materials and methods: Data from 586 patients starting DRV/r (195 protease inhibitors [PI]-naïve, 391 PI-experienced) in combination with at least two antiretrovirals were examined. Among patients for whom the dosage was known (N=236), 28% were treated with DRV600 twice daily. All patients analyzed had a baseline viremia >50copies/mL and an available baseline genotypic resistance test (GRT). Kaplan-Meier method was used to estimate the probability of both virological success (VS, viremia<50 copies/mL) and virological failure (VF, the first of two consecutive viremia-values >200 copies/mL, after six months of pressure), according with the baseline viremia and the number of DRV-resistance mutations. In a subset of 108 patients failing DRV/r with an available GRT both at baseline and at failure (19 PI-naïve, 89 PI-experienced), the emergence and the level of DRV-resistance was evaluated.

Results: Overall, the median [Interquartile range] baseline viremia was 4.6[3.5-5.3]log10 copies/mL (PI-naïve patients: 5.0[4.3-5.5]log10 copies/mL; PI-experienced patients: 4.4[3.0-5.1]log10 copies/mL; p<0.001). By 12 months of therapy, the probability of achieving VS was 87%. No significant difference in the probability of VS was found by stratifying for the number of DRV-mutations at baseline (p=0.941). The median time [95% Confidence Interval] to achieve VS was similar between PI-naïve and PI-experienced patients (4.0[3.7-5.0] vs. 3.4[3.0-4.0] months), although the probability to achieve VS by 12 months of DRV/r-therapy was higher in PI-naïve patients (94% vs. 83%, p=0.041). By stratifying for baseline viremia, in PI-naïve patients the median time to achieve VS significantly increased by increasing viremia levels (<100,000copies/mL: 3.2[2.8–3.7] months; 101,000-500,000: 5.0[4.2–6.4]; >500,000: 5.5[4.4–8.8], p=0.003). However, the rate of success by 12 months of treatment was similar in all viremia-ranges (93% vs. 94% vs. 96%). By 24 months of DRV/r-treatment, the rate of VF was 23%. As expected, the rate of VF was significantly lower in PI-naïve than in PI-experienced patients (11% vs. 28%, p<0.001). The analysis of the GRT pairs (baseline-failure) from patients who had failed DRV/r showed no emergence of DRV-mutations in PI-naïve patients. In the 89 PI-experienced patients, 62 (69.7%), 16 (18.0%) and 11 (12.3%) patients had low, intermediate, and high resistance to DRV, respectively. A significant increase in the prevalence of the DRV-mutations V32I (from 12.3% to 21.3%, p=0.021), I47V (from 5.6% to 14.6%, p=0.008), I50V (from 0% to 6.7%, p=0.041), L89V (from 11.2% to 19.1%, p=0.016) and the major PI-mutation M46I (from 22.4% to 32.6%, p=0.022) was found at failure.

Conclusions: In an unselected population of HIV-infected individuals, receiving DRV/r as part of antiretroviral-regimen results in effective virological-response both in PI-naïve and PI-experienced patients. High success rate was obtained also in patients with high viral load at baseline. Rate of resistance to PIs is nearly zero at first line failure, and modest in PI-experienced patients treated with darunavir.

No conflict of interest

Abstract: P_26

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Minimal CD4 gain and high PR/RT resistance rate in patients failing Raltegravir with 148HKR pathway

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Background: We evaluated whether the various pathways of resistance mutations (RAMs) observed in raltegravir failing patients differentially affect viro-immunological parameters.

Methodology: HIV-1 infected patients failing a raltegravir containing regimen in Italy and Spain were analyzed. Baseline genotypic sensitivity score (GSS), HIV-RNA, CD4 cell count, therapy associated with raltegravir, and resistance in protease (PR) and reverse transcriptase (RT) were investigated according to raltegravir resistance.

Results: Among 176 raltegravir-failing patients, 5 were on first-line HAART and 171 were HAART-experienced (10% of patients with HIV-RNA≤50 copies/mL). The median [IQR] baseline CD4 cell count was 223 [117-407] cell/mm^3, and the median [IQR] baseline HIV-1 RNA was 4.3 [2.9-5.0] $\log_{10}$ copies/mL.
At failure, 64% of patients failed with at least 1 raltegravir-RAM. The most common resistance pathway included N155H mutation (26%, often associated with T97A-V151I-G163R-E92Q), followed by Q148HKR (18%, often associated with G140SA-E138AK) and Y143CHR (10%, often associated with T97A-G163R). 36% of patients failed without raltegravir-RAMs, while 8% of patients with only secondary RAMs and 2% with mixed primary RAMs. No significant differences in terms of baseline CD4 cell-count, HIV-RNA and GSS were observed among different resistance pathways or no-resistance. Patients failing with HIV-RNA>1000 copies/mL (N=129) showed a higher frequency of primary raltegravir-RAMs compared with those (N=47) having HIV-RNA≤1000 copies/mL (61% vs. 40%, p=0.014). Regarding the specific resistance pathways, only Y143-pathway showed a significant higher frequency in patients with HIV-RNA at failure >1000 copies/mL (13%) compared to those with HIV-RNA≤1000 copies/mL (3%) (P=0.046). On the other hand, the presence of N155H, or Q148HKR, or only secondary RAMs was statistically independent of HIV-RNA level at failure. Moreover, patients harboring N155H or no primary RAMs showed a significant CD4 cell gain at failure (median [IQR] CD4 cell gain: no primary-RAMs, 56 [-35;177] cells/mm^3, p<0.001; N155H, 69 [-50;171] cells/mm^3, p=0.015). Differently, patients failing with 143 or 148 resistance-pathway did not show a significant increase of CD4 cell count during raltegravir-treatment (median [IQR] CD4 cell gain: Y143CHR, 57 [7;169] cells/mm^3, p=0.148; Q148HKR, 10 [-11;81] cells/mm^3, p=0.242).
Regarding PR/RT resistance at raltegravir-failure, the highest prevalence of 3 classes resistance was observed in patients failing with 148-pathway (148 vs 155 vs 143 vs no primary RAMs: 48% vs 19% vs 19% vs 11%, p<0.001). Conversely, the highest prevalence of no resistance in PR/RT was observed in patients failing in absence of primary raltegravir RAMs (no primary RAMs vs 155 vs 143 vs 148: 53% vs 27% vs 12% vs 12%, p<0.001).

Conclusions: Among raltegravir failing patients, less than 20% failed with the Q148HKR resistance pathway. These patients frequently showed minimal CD4 cell count gain and high resistance to the other classes of antivirals. In these compromised failing patients, particular attention should be focused

for the best tailoring of their subsequent HAART regimen.

No conflict of interest

Abstract: P_27

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Complex antiretroviral drug resistance mutation profiles from South African children failing a 2nd-line regimen

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Background: Data on antiretroviral drug resistance mutation patterns of children failing 2nd-line regimens in South Africa is scarce. This study characterizes HIV-1 drug resistance patterns in a subset of children failing didanosine -containing2nd-line regimens in South Africa.

Materials and Methods: A retrospective analysis was performed on HIV-1 genotype profiles of 65 children, with proven virological failure on 2nd- line regimen. Samples were referred from regions within South Africa and processed at the Charlotte Maxeke Johannesburg Academic Hospital HIV genotyping laboratory during the period July 2009 to July 2012.

Results: Patients had a median age of 12 years and consisted of 53% males (n=35). Median total time on ART was 48 months (IQR: 31.5-60 months). Forty percent of patients were receiving specialist tertiary level paediatric care while the rest were attending district/ regional level facilities. Median viral load was 4.72 log 10 RNA copies/ml (IQR: 4.1 log 10 -5.32 log 10 RNA copies/ml).Median CD4 count was 436 cells/mm^3 (IQR: 152 -837 cells/mm^3).
Fifty-three patients failed a PI + two NRTI regimen ((82%), whereas 12 (18%) patients failed a NNRTI + two NRTI regimen. Seventy-three percent (n=50) of those with virological failure had evidence of antiretroviral drug resistance. Seventy-eight percent of samples (n=51) had NNRTI resistance with K103N and G190A the most commonly present mutations, both in 15% of samples. Despite 93% of the 65 patients having evidence of either d4T or AZT exposure, only 1 patient (1.5%) had ≥ 3 TAMs. All patients had a history of lamivudine exposure butM184V was present in only 13% of cases. Two patients were exposed to lamivudine at the time of resistance testing. Forty-nine percent of patients remained susceptible to didanosine; and 53% to abacavir. Susceptibility to the remaining NRTIs varied between 60-67 %. NNRTI susceptibility profiles were low at 33% for EFV and 22% for NVP. Etravirine and rilpilvirine susceptibility were 69% and 63%, respectively.
The time patients were exposed to PI's during 1st- or 2nd-line regimens did not differ (median 20 months). Only 12% (8/65) of patients with current or prior PI-exposure developed major protease inhibitor mutations, half of which (n=4) had current PI-exposure. The most common major PI mutations present were I54V (n=7) and V82A (n=7). Future PI options are limited as only 50% (n=4) remained fully susceptible to darunavir and 37.5% (n=3) to tipranavir.

Conclusion: Sixty percent of patients were not receiving specialist paediatric care. The complexity of drug resistance patterns observed suggests that this required. This recommendation is based on: most patients still being susceptible to PI's, challenges in predicting development of PI and NRTI resistance; and extensive PI cross-resistance profiles. NNRTI susceptibility is mostly limited to etravirine and rilpilvirine, drugs that are not freely available in South Africa and limited by age restriction. Clearly, paediatric patients on 2nd-line ART therapy in South Africa should be carefully monitored and referred to tertiary facilities speedily, if and when, virological failure occurs.

No conflict of interest

Abstract: P_28

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

How impaired is the selection of a powerful treatment regimen in pre-treated HIV patients?

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Background: ART-naive patients usually have a broad range of ARVs available to choose the most appropriate combination for a fully active response. In contrast, Treatment-experienced patients have only limited options. Very few data are available to what extent therapy options are impaired for pre-treated patients on regimen level and how new drug classes might facilitate the assembly of potent regimens for them.

Methodology: Retrospective, anonymized, epidemiological study in two German databases (Arevir and mib DB). Inclusion criteria were HIV-1+ adults with last documented treatment episode of ≥ 6 months who stopped treatment due to VF (VL >50 c/ml) since Dec. 31, 2007. Genotypic sensitivity score (GSS) was calculated using the HIV-GRADE algorithm if genotypic resistance testing was available. Activity of a drug class is derived from the drug with the highest score in the class or the sum of two drugs in case of NRTIs. cART was classified sufficient with a score-sum ≥ 3. Previous virological failure of an InI containing regimen was classified as a score of 0, due to missing integrase resistance testing. This is expected to overestimate InI resistance. PI/r and InI combinations are intensively investigated as treatment option in pretreated patients. In case of fully active PI and InI the combination was counted as adequate treatment option.

Results: More than 11,000 patients are documented in our DBs, which represents 15% of the German HIV population. 2352 of these patients met the selection criteria and in 630 patients genotypic resistance data could be evaluated.
According to the recommendations of different guidelines, regimens in patients failing a previous regimen are still based on the combination of 2 NRTI + 3rd agent, although different NRTIs and a 3rd agent from a different class should be preferred. Comparing different combinations of 2 NRTI + 3rd agents, a combined score ≥ 3 could not be reached in 15% of patients with 2 NRTI + PI (2 NRTI + NNRTI: 20%). 2NRTI + InI also showed insufficient scores for 20% of patients. Based on our assumptions on InI activity, this is a worst case scenario and overestimated due to the high rate of 9.6% of previous InI treatment. If dual treatment with InI and PI is considered an option, only 3.1% of patients show insufficient scores. In quadruple treatments still 4.9% of GSS scores are below three and classified as insufficient.

Conclusions: Despite over 20 licensed ARVs, treatment options are clearly impaired for patients with previous VF. Up to 20% of the patients cannot be treated with a sufficiently potent standard regimen. The new drug classes like InI and strategies such as dual PI/InI can improve this problem. Nevertheless a small amount of patients still is left without sufficient treatment options and further development of new drugs remains important.

Conflict of interest: Study sponsored by ViiV Healthcare

Abstract: P_29

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

HIV-1 drug resistance tests during low-level-viremia: help and hindrance

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Background: Modern highly active continuous antiretroviral therapy (cART) leads to sustained suppression of viral replication in the majority of patients. The repeated detection of HIV-1 RNA in the plasma is in general the major indicator for treatment failure and evolution of drug resistance. In contrast the singular detection of low levels HIV-1 RNA in patients with usually well-suppressed viral loads, called blips, is not associated with disease progression or development of drug resistance. Especially in patients with low-level-viremia the early detection of ongoing viral evolution towards HIV drug resistance can be challenging but would be highly beneficial. Therefore we scrutinized the performance of two genotypic HIV-1 drug resistance tests in these patients and compared these results with genotypes obtained from proviral DNA.

Material and Methods: All samples were obtained for genotypic HIV-1 drug resistance tests in routine diagnostics and tested for viral RNA (m2000 real time system, Abbott Molecular). Only samples from patients treated with cART and viral loads below 1000 copies/ml were included in this study (n=46). HIV-1 drug resistance tests were performed using both an in-house method and the TRUGENE HIV-1 Genotyping Assay, Siemens. Proviral DNA was analyzed using the in-house method only.

Results: The HIV-1 drug resistance test of viral RNA yielded results in 40 (86.9%) samples with an average viral load of 256(+/-222) copies/ml. In five cases only proviral DNA could be analyzed (71(+/-12) copies/ml) and in one case neither RNA nor DNA could be genotyped (47 copies/ml). The majority of patients (n=31, (68%)) were treated with boosted PIs at the time of low-level-viremia (318(+/-248) copies/ml).
HIV-1 genotypes obtained from viral RNA were either wild-type (Wt) (n=21, 274 (+/-246) copies/ml) or harbored drug resistance mutations (n=18, 237(+/-199) copies/ml). In the RNA Wt group three proviral DNAs carried drug resistance mutations ((PR) M46I, (RT)M184V, (RT)M41L/(PR)M46I/L90M). In the RNA drug-resistance group to some extend the RT mutation M184V was not detected in DNA (n=4), and once even not in a previous genotype. In two cases RNA and DNA genotypes carried RT mutations (K103N/M184V, K103N), which were not present in previous genotypes.
In 14 cases HIV-1 genotypes from RNA could only be analyzed with the TRUGENE assay (174(+/-132) copies/ml) revealing 9 wild-type and 5 drug resistant viruses (RT: K103N, E138A, M184V (n=3)). In contrast only one plasma sample could solely be analyzed with our in house method. Interestingly even HIV-1 isolates which could be amplified and sequenced by both systems, partly showed different genotypes in both assays (M41ML-D67DN-M184MV-L210LW-T215NSTY vs. D67DN-L210LW-T215NSTY; M41L-K101E-L210W vs. M41L-L210W; (PR)L23I-D30N-M46I-F53L-I54L-Q58E-N88D-(RT)K65KR-L74LV-M184V vs. (PR)D30N-N88D)

Conclusions: Low-level-viremia can indicate ongoing viral evolution towards antiretroviral drug resistance but most often no evidence of emerging viral drug resistance can be detected. HIV-1 drug resistance assays can vary in their diagnostic sensitivity at times of low-level-viremia and even mutational clusters can differ in these settings due to the amplification of different HIV variants from the same sample. In the future additional parameters might help to identify patients with ongoing viral replication more precisely.

Conflict of interest Study was partly supported by Siemens diagnostics.

Abstract: P_30

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Comparative drug ranking for clinical decision support in HIV treatment

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Background: Despite the impressive body of research and the huge amount of data available on both the virology and the clinical aspects of the HIV infection, HIV-related health care is still far from an optimal evidence-based care. Currently the three most used drug resistance databases, HIVdb, REGA and ANRS, are expert based systems and for this reason their drug ranking tools are prone to discordances. Selecting the most appropriate drugs given the specific genome of the prevalent viral strain to be treated is extremely important, since a suboptimal choice in the early phase of the infection might severely compromise the life expectancy of the patient. This issue can be tackled by interdisciplinary research combining medical and biological data with advanced computational science methodologies. We present an innovative Comparative Drug Ranking System (CDRS), which identifies drug ranking discordances and provides tools to rapidly take an informed decision.

Materials & Methods: Our tool compares the rankings of each system using first-order logic to clean rules, identify discordances and check their logical consistency. It automatically downloads updates in the rules of each drug resistance database, in order to be always up-to-date. CDRS can efficiently identify and display eventual discordances and drug interactions. In case of discordance between the expert systems CDRS allows the clinician to assess its entity and to display the individual mutations that generated it. Using innovative literature mining tools and the access to our database of relevant scientific publications we provide evidence, always up to date, to reduce the uncertainty and take an informed decision to resolve the discordance.

In order to assess how often the use of our system would help to resolve potentially dangerous discordances, we evaluate the occurrence of discordances between the three expert based systems using clinical data on 11795 patients from the Euresist database. The patients used in this study were selected based on the completeness of the genotypic data. We selected only those patients who had the HIV genotypic data for both reverse transcriptase and protease. For each antiretroviral drug we categorize such discordances into minor and major discordances according to the differences of the drug rankings provided by each system.

Results: Our analysis shows that for 56,26% of the 11795 patients from the EUresist database there are both at least one major and one minor discordance in at least one drug class.

Conclusions: The CDRS offers an opportunity to improve the quality of clinical practice and to reduce the emergence of drug resistances in HIV infected patients by supporting the clinicians with an evidence-based decision tailored to the needs of the specific patient.

No conflict of interest

Abstract: P_31

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Primary Resistance to Integrase Strand-Transfer Inhibitors in Europe

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Background: Primary HIV-1 resistance can compromise the efficacy of antiretroviral therapy (ART). The prevalence of primary resistance to integrase strand-transfer inhibitors (INSTI) in recently HIV-1-infected subjects remains unknown.

Methodology: This was a multicenter cross-sectional study within the European SPREAD surveillance programme which continuously collects data from a representative set of newly diagnosed patients. A representative set of 300 samples from 1950 patients diagnosed in 2006/2007 was selected from the main cohort. The prevalence of INSTI resistance was evaluated using quality controlled baseline population sequencing of integrase (IN). A subset of 20% were further evaluated using 454 IN sequencing. For both analyses, we assessed major mutations based on the IAS-USA mutation table. All integrase substitutions were classified according to their Stanford HIVdb score ≥10 for at least one INSTI. Minority variants (MV) were defined as substitutions present in 1-20% of viruses in the 454 analysis. Subtypes were determined from population sequencing data using the Rega Subtyping Tool vs 2 based on pol.

Results: For the population sequencing analysis, 279 samples were retrieved and successfully analysed. 67% of isolates were subtype B. No signature resistance mutations to INSTIs (N155H, Q148H/R/K Y143R/H/C) were detected. In 11 (4%) patients the following mutations on resistance associated positions were detected with HIV db score ≥10: L74M (n=2; 1%), T97A (n=2; 1%), E138A, A153F, E157Q (n=2; 1%), G163KT, R263K, V151I + G163EKR. In 31 (11%) patients mutations with HIVdb score <10 were found: L68I, L68V, L74I (n=13; 4.6%), L74V, T97S, A128T (n=2, 1%), E138D (n=2; 1%), E138G, G140W, V151I (n=6; 2%), 68V + L74V, 143DN + Q146E. Subsequently 56 samples were analysed with 454 sequencing. Median overall coverage was 4294 reads/substitution. Again, no signature mutations were detected. IN substitutions with HIVdb score ≥10 were found in 7 (12.5%) subjects: E157Q in 5 (8.9%) subjects, 1 as MV; H51Y and G163R in 1 (1.7%) subject each, both as MVs; and G163K in 1 (1.7%) individual. IN mutations with HIV db score <10 were: V151I in 6 (10.7%), 3 as MVs; L74I in 3 (5.3%), 1 as MV; and A128T, N155D, H51Q and T97I in 1 (1.7%) subject each, all as MVs.

Conclusions: As expected, no signature INSTI-resistant variants were circulating in Europe before the introduction of INSTIs, either as high- or low-frequency variants. However, polymorphisms contributing to INSTI resistance were not rare. As INSTI use becomes more widespread, continuous surveillance of primary INSTI resistance is warranted. These data provide a baseline for that purpose and will be key to model the kinetics of INSTI resistance transmission in Europe in the coming years.

No conflict of interest

Abstract: P_32

Mechanism of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Model to investigate host-specific innate and adaptive immune responses in therapy-naïve HIV-1 infected patients

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Background: Understanding host-specific innate and adaptive immune responses in therapy-naïve HIV-1 infected patients is of high importance. In a drug-free environment, the immune system is the most dominant factor shaping the evolution of HIV-1. Mutations can be selected, causing the adaption of the virus to the HLA alleles of the infected individuals, negatively influencing the immunogenicity of epitopes. However, in treatment-experienced patients, the most dominant factors are antiretroviral drugs. Drug resistance associated mutations (RAMs) can evolve which can be transmitted (tRAMs) from one individual to another. It is assumed, that a persistence of tRAMs in therapy-naïve patients is influenced by the HLA alleles of the new host and that persisting tRAMs are most likely located within HLA class I restricted epitopes, leading to immune escape. Here, a cell culture model was established to identify a direct impact of tRAMs on host-specific innate and adaptive immune responses in the background of certain HLA types.

Materials &Methods: On the basis of a statistical analysis, identifying nine significant associations between PI, NRTI and NNRTI tRAMs and the patients' HLA class I alleles, a cell culture model was established. Immature monocyte-derived dendritic cells (moDCs) were transduced using lentiviral vectors to introduce HLA alleles of interest, here HLA-A*02:01. Subsequently, these moDCs were infected with recombinant HIV-1 ADA, harbouring the tRAMs to be investigated, here V82A in the protease. To study the impact on both, the innate as well as the adaptive immune system, infected recombinant moDCs were co-cultured with either $CD16^+CD56^+$ NK cells or $CD8^+$ T cells, in order to determine cytokine and chemokine release in a final step.

Results: Nine significant associations between tRAMs and HLA class I alleles could be identified in a cohort of 139 therapy-naïve patients. MoDCs were successfully transduced to express HLA-A*02:01 and could be infected with the recombinant HIV-1 ADA, containing V82A. Viable $CD16^+CD56^+$ NK cells and $CD8^+$ T cells were purified to use in the co-cultures. To determine an impact of tRAMs on epitope immunogenicity and to identify potential biomarkers to monitor immune activation regarding immune escape, cytokine and chemokine levels can be evaluated. So far, IL-8 could be identified as a potential candidate.

Conclusion: Interactions of immune system and antiretroviral treatment could especially be important in the treatment of therapy-naïve patients already harbouring tRAMs. The consideration of HLA allele mediated immune selection could exclude antiretroviral drugs promoting a therapy failure by providing a selective pressure for tRAMs conferring not only resistance to the drug but also immune escape. Treatment could be optimized using all available information regarding virus and host to facilitate a long lasting therapy success.

No conflict of interest

Abstract: P_33

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

HBV immune/vaccine escape mutations in Polish chronic hepatitis B patients treated with nucleos(t)ide analogues.

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Background: Hepatitis B virus (HBV) polymerase (*pol*) gene overlaps with surface antigen (HBsAg) encoding region (*S*). The HBsAg synthesis can be altered by *pol/S* gene mutations. The selection of genetic variants is triggered by exposure to anti-HBs immunoglobulins or by nucleos(t)ide analogues (NAs) pressure on *pol,* inducing resistance mutations. These HBV *pol/S* gene overlap mutants are named vaccine-escaped mutants (VEMs) or antiviral drug-associated potential

vaccine-escaped mutants (ADAP-VEMs). Changes in amino acid sequence occurring especially within Major Hydrophilic Region (MHR) of the 'a' determinant could reduce the antigenicity and immunogenicity of the HBsAg. The failure of HBsAg neutralization allows HBV escape from the host's immune system resulting in continued active viral replication, liver disease progression and potential infection in vaccinated individuals. The main ADAP-VEMs are recognised and are the object of presented study.

Objectives: The aim of the study was to investigate the prevalence of HBsAg immune/vaccine escape variants among patients with chronic hepatitis B (CHB) undergoing NAs treatment, by population sequencing of *pol/S* gene.

Material and methods: The serum samples from 600 persons [211 (35,1%) women and 389 (64,9%) men] with CHB admitting infectious diseases clinics were tested in order to determine immune/vaccine escape HBsAg mutants. HBV DNA was isolated using the GeneMatrix QuickBlood DNA Purification Kit (EURx, Poland). The *pol/S* region was amplified by PCR. Direct sequencing of ~1kb amplicon was performed using BigDye Terminator Cycle Sequencing Kit v3.1 and ABI Prism 3130 Genetic Analyzer (Life Technologies). Obtained sequences were analyzed by the Seqscape 3.1 software (Life Technologies) and interpreted with geno2pheno algorithm (Max Planck Institut).

Results: Overall in 166 (27,6%) of tested samples VEMs associated with 1 and 2 loop of 'a' determinant HBsAg were recognised. Among them 201 single mutations were distinguished. The prevalence of most frequent escape variants was as follow: s128V (n=55;27,4%), s120T/S/A (n=27;13,4%), s133T/I/L (n=21;10,4%), s130N/R/D (n=13;6,5%), s145R/K/A (n=10;4,9%), s126I/N/S (n=10;4,9%) and s110M/V (n=9;4,5%). No differences between NAs resistant or susceptible strains in mutations prevalence in analysed region were detected. In samples with genotype D2 genetic variant s128V was predominant, occurring in 54 (98,1%) of cases. The s120T/S/A and s133T/I/L mutants were common in genotype A2 samples with prevalence respectively 23 (85,2%) and 17 (80,95%).
Additionally the strict ADAP-VEMs were found in 225(37,5%) of cases, which gave 294 point mutations. The most frequent mutations profile, corresponding to reduced susceptibility for the particular NAs, were found as listed:

*lamivudine (n=156;53,1%) - s195M (n=89;30,2%), s196L (n=41;13,9%), s164D (n=19;6,5%);
*entecavir (n=79;26,9%) - s195M (n=31;10,5%), s161H (n=19;6,5%), s175F (n=12;4,1%), s164D (n=9;3,1%);
*adefovir (n=5;1,7%) - s172F (n=2;0,7 %) and s173F (n=1;0,3%).

In strains interpreted as susceptible (n=54;18,4%) we detected: s161F (n=21;7,1%), s194V (n=19;6,5%), s195t (n=6;2%).

Conclusions:
1) Immune/vaccine escape variants of HBV are widespread among NAs-experienced patients.
2) ADAP-VEMs are most common in group of lamivudine-treated patients; this might be the result of long-term lamivudine therapy.
3) Overlapping structure of HBV genome modulates the extent of HBV genetic variability.
4) Avoiding the emergence of antiviral drug resistance is crucial for preventing transmission and spreading of immune/vaccine escape HBV mutants.

No conflict of interest

Abstract: P_34

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Emergence of drug resistance during long-term persistent low-level viremia under ART

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Background: After the introduction of HIV-1 viral load assays with a lower quantification limit of 20 copies/ml (c/ml), an increased number of patients showed low but systematically detectable viremia while receiving antiretroviral therapy (ART). The causes and clinical

consequences of this persisting low-level viremia (PLLV) remain elusive and few data are available on the potential emergence of drug resistance in these patients. Our objective was to analyse the emergence of drug resistance during PLLV in HIV-1 infected patients on ART.

Material & Methods: Patients with PLLV were retrospectively selected based on plasma viral load (pVL) measurements between March 2009 and December 2012. PLLV was defined as a pVL between 20 and 250 c/ml in at least half of minimum 6 pVL measurements from 6 months after the initiation of therapy onwards. Resistance mutations in the *protease* and *reverse transcriptase* genes were assessed at the initiation of ART (baseline). An ultrasensitive sequencing assay was developed to define resistance mutations in the low level viremia samples collected longitudinally after ART initiation.

Results: 11 patients were selected for baseline resistance testing and longitudinal analysis of drug resistance during PLLV. Patients were followed for a mean period of 57.3 ±16.2 months. All patients were male and were infected with a subtype B virus, nine patients were treatment-naive at ART initiation. Mean viral load during PLLV was 65.3 ±21.2 c/ml. Current ART regimens contained 2 NRTI (100.0%), boosted PI (81.8%), NNRTI (18.2%), raltegravir (9.1%) and maraviroc (18.2%). Eight patients switched ART during longitudinal follow-up. No drug resistance mutations (DRMs) were detected in baseline samples. A total of 137 samples collected during PLLV were analysed and a genotype was obtained for 81 (59.1%). The mean viral load of the 137 samples was 55.3 ±47.4 c/ml, 26 (19.0%) samples were reported as having less than 20 c/ml. Except for the NNRTI resistance associated K101E mutation in one sample, no DRMs were detected. The patient with the K101E substitution was receiving an EFV based ART regimen, but switched to a PI based regimen after the emergence of the DRM. The mutation was no longer detected in samples collected after the regimen switch.

Conclusions: None of the 11 tested patients harboured baseline DRMs, and 10 patients showed no evidence of selection of DRMs during a long period of PLLV. These results suggest that selection of DRMs was neither cause nor consequence of PLLV. The detection, in one patient, of an NNRTI resistance associated mutation that was not present in previous samples, however, indicates that resistant variants can emerge at viral loads below 250 c/ml.

No conflict of interest

Abstract: P_35

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Protease diversity and resistance to protease inhibitors of viruses archived in peripheral blood mononuclear cells of HIV-2 infected patients

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Introduction: Currently, there is no information on protease diversity and susceptibility to protease inhibitors of viruses archived in peripheral blood mononuclear cells (PBMCs) of HIV-2 infected patients. Response to treatment and initial treatment options may be conditioned by these viruses. The aim of this study was to characterize protease diversity and resistance to protease inhibitors of isolates present in PBMCs of treated and untreated HIV-2 infected individuals.

Material & Methods: Blood samples were collected from 26 HIV-2 infected patients attending Hospital de Santa Maria, Lisbon. Fourteen patients were on antiretroviral treatment (the majority with AZT, 3TC and LPV/r) and 12 patients were untreated. CD4 count was lower in the treated patients compared to untreated patients (315.7 cells/mm^3±204 vs 780.4±442; P=0.0031. Of the 8 patients with viral load data, 3 (37.5%) had high viral load (range: 10,425-100,000 cp/ml) and 5 had undetectable viral load. A 363 bp DNA fragment corresponding to the PR protein

was amplified by PCR from proviral DNA present in the chromosomal DNA extracted from the peripheral blood mononuclear cells (PBMCs) of all patients and cloned into pCR4-TOPO®. Plasmid DNA was extracted and a mean of 7 clones per patient was sequenced. PIs resistance mutations were identified using HIV-Grade. Phylogenetic analysis was performed by maximum likelihood methods using the best fit model of evolution and bootstrap resampling.

Results: In total, 93 clonal sequences were generated from 14 treated patients and 87 from 12 untreated patients. All patients were infected with group A viruses. Mean genetic distance between patients was 0.106 number of substitutions/site ±0.025 and within patients was 0.019±0.012. Genetic distance between treated patients was significantly higher than between untreated patients (0.1110± 0.02300 vs 0.09897± 0.02262, P=0.0036).
PR mutations previously associated with resistance to PIs were detected in 21 (22.6%) sequences from treated patients (7 patients) and in 2 (2.3%) sequences from 2 untreated patients (P<0.0001). Resistance mutations were present in all sequences from 2 treated patients; in the remaining patients the proportion of clonal sequences harboring resistance mutations was low (range, 10% to 36%). Frequency of resistant clones was residual in the 2 untreated patients (10%).
The most common PI resistance mutation in treated patients was I84L (n=4; 4.3%). Other resistance mutations were I54M and I82F (n=3; 3.2%), I50T (n=2; 2.2%) and G48R (n=1; 1.1%). V7II and I89V substitutions were detected in clonal sequences from treated and untreated patients while L99F was only detected in treated patients. These substitutions may act as accessory mutations associated to PI resistance. There was no association between the presence of resistance mutations and CD4 number or viral load.

Conclusions: Half of the HIV-2 patients on treatment have protease resistant isolates in PBMCs irrespective of the result of therapy. The impact of these archived viruses on the long-term response to antiretroviral treatment needs to be determined. The high prevalence of antiretroviral naïve patients infected with drug resistant isolates indicates that clonal sequencing of proviral DNA may be important to guide the starting drug regimen.

No conflict of interest

Abstract: P_36

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Phenotypic analysis of patients on current anti-HCV Therapy

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Background: In published studies approximately 50% of Hepatitis C Virus (HCV) infected patients, who do not achieve sustained-virological-response (SVR), displayed resistance associated mutations (RAMs) to Boceprevir (BOC)/Telaprevir (TVR). The aim of this non-interventional study is to phenotypically analyse samples from patients failing therapy with/without detected RAMs, or achieving SVR with RAMs.

Methods: Bicistronic subgenomic genotype 1b replicons encoding for Firefly Luciferase under the control of a polio virus IRES and the non-structural HCV proteins NS3 to NS5B under control of an EMCV IRES had been modified to generate NS3-protease-libraries from selected patients. After transfection of Huh-7-LUNET cells BOC/TVR drug titration was performed and after 68h incubation a luminescence-based readout was conducted to determine IC50 and IC90 values of the respective replicon libraries. These data were statistically evaluated and compared to geno2pheno$_{[HCV]}$ predictions.

Results: 2/4 week-0-samples (RAMs: 54S;36L) showed resistance profiles. Both patients had TVR-treatment (discontinuation; SVR). In 2/4 week-0-samples (168G;117H) with RAMs no resistance but minor increase in viral-fitness was observed. One BOC-therapy-patient

developed the 36A mutation during lead-in-phase and resistance was phenotypically confirmed by elevated IC50/IC90 values. All three therapies are currently ongoing (VL<12IU/mL). One BOC/TVR-susceptible week-12-sample (TVR-therapy) showed slight increase in viral fitness.

Conclusion: Genotypic resistance prediction with geno2pheno[HCV] was phenotypically confirmed. By collecting phenotypic data from NS3 libraries we will continuously improve the geno2pheno[HCV] algorithm to guarantee effective therapy regimes.

No conflict of interest

Abstract: P_37

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Analysis of NGS data from heterogeneous HCV1b populations

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Background: The analysis of next-generation sequencing (NGS) data from heterogeneous viral populations such as hepatitis C virus (HCV) remains a non-trivial exercise. In order to accommodate the specific characteristics of short read fragment data derived from such diverse populations, we studied the impact of tailored analysis methodologies and of reference sequence divergence on the recovery of minority variants.

Materials and methods: Four samples from treatment-naïve HCV1b infected patients were amplified by nested PCR and sequenced with Illumina's Genome Analyzer IIx. The performance of five software packages (MAQ, Bowtie, BWA, Velvet and Segminator II) for aligning the quality trimmed Illumina reads against a reference sequence was tested. To assess the impact of using a related reference sequence, all reads of each sample were mapped to a published HCV1b reference sequence (GenBank: AB049087), to a contig of the same sample obtained by Sanger sequencing and to an in silico reconstructed data-specific reference sequence (VICUNA and V-FAT). Concordance between the viral population obtained after Sanger sequencing and after read mapping with Segminator II using the three mentioned reference sequences, was compared. The observed differences in minority variants between the analysis with a sample-specific and a more distantly related sequence were compared. Positions with high difference (>5% threshold) were compared with the location of high divergent regions, defined as areas with Shannon entropy above the 75th percentile.

Results: The number of mapped reads was consistently higher when a sample-specific sequence was used instead of the standardly used but more divergent HCV1b reference sequence. For the different software packages, recovery of reads varied between 20% and 82% with Segminator II performing best: 80.6% (± 3.0) against the sample-specific Sanger sequence, 77.3% (± 2.4) against the Genbank reference sequence (using the automatic cyclic remapping), and 82.1% (± 4.0) against the in silico reconstructed reference sequence. Simple scoring of aligned positions as matched/unmatched revealed a high degree of concordance between the NGS consensus sequences obtained by Segminator II (all three reference sequences) and the sample-specific sequence obtained with Sanger sequencing. After neglecting the minor discordances (predominantly attributable to ambiguity characters in the NGS consensus sequences), overall concordance increased to a nearly perfect match (>99%). However, the use of the standard HCV1b reference sequence resulted into slightly higher discordance values (0.30%) as for the Sanger (0.026%) and in silico reconstructed reference sequence (0.05%). Although the main differences in minority variants were located below the 0.5% threshold, in total 184 differences at frequencies above 5% were observed. All these positions were located at (70.34%) or within 5 nucleotides of a divergent area.

Conclusions: Only a few software packages can accommodate the diversity present in heterogeneous viral populations, and using a distantly related reference sequence leads to substantial data losses, even with the best among the software packages, Segminator II. Although the viral population of the three reference sequences did not differ substantially, discordances were enriched in highly divergent regions. So we recommend the use of a sample-/data-specific reference sequence for read mapping.

No conflict of interest

Abstract: P_38

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Hepatitis C Virus Screening Project of Patients on current anti-HCV Therapy

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Background: Clinical outcome of HCV genotype-1 therapy combination of pegylated-interferon-alpha (IFNα), ribavirin (RBV) and protease-inhibitors (PIs) depends on host and viral factors. This non-interventional study collects data from PI-resistance-associated-mutations within the NS3 gene, viral quasispecies distribution and host factors, in order to predict clinical outcome using the geno2pheno$_{[HCV]}$-tool.

Methods:NS5B+NS3 sequences from plasma samples were obtained. Subtyping (including GT-1a samples clade classification) and resistance against Boceprevir (BOC) and Telaprevir (TVR) was determined with geno2pheno$_{[HCV]}$ (http://hcv.bioinf.mpi-inf.mpg.de/). If possible, host IL28B-polymorphism was tested.

Results:130 GT1-infected patients are up-to-date enrolled. IL28B polymorphism was available for 121 patients: 29xCC, 75xCT, 17xTT.
74/130 samples were 1a and 56/130 1b. Baseline BOC/TVR-resistance-associated-mutations were detected in 22/130 (16.9%) baseline-samples: 9x132V, 4x117H, 1x132V + 174F, 1x168G, 1x36L, 1x36A, 1x36M+80K+ 155K, 2x54S, 1x54S+80R, 1x54S+155K.
73 GT 1a-samples could be further classified: 44/73 clade-I and 29/73 clade-II. The mutation Q80K, conferring treatment problems with simeprevir, was observed in 20/35 clade-I baseline samples.

Conclusion: Analysis of NS5B+NS3 with geno2pheno[HCV] interpretation allows subtyping, clade classification, and prediction of PIs susceptibility. PI resistance-mutations exist at baseline in higher extent as reported in previous studies. Incorporating additional viral and clinical data will continuously improve geno2pheno$_{[HCV]}$ for better therapy response prediction.

No conflict of interest

Abstract: P_39

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Dolutegravir and Elvitegravir resistance mutations in HIV-infected patients failing Raltegravir

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Background: Elvitegravir (EVG) and Dolutegravir (DGV) are the most recently approved integrase inhibitors. They depict different antiviral activity against isolates from patients who have failed on raltegravir (RAL). However, changes at codon 148 seem to impair both EVG and DGV susceptibility although with different extent.

Methods: All patients on RAL therapy attended from January 2008 to December 2012 at one large reference HIV clinic located in Madrid were identified. Integrase sequences were obtained from all patients who failed on RAL. The prevalence of EVG and DGV resistance associated mutations (RAMs) as well as the proportion of estimated EVG- and DGV-resistant individuals, as reported using the Spanish resistance algorithm (www.retic-ris.net) were assessed.

Results: RAL prescription at our centre increased from 305 in 2008 (17.2% of patients on antiretroviral therapy) up to 641 (32%) in 2010 (p<0.0001), 638 (29.7%) in 2011 and 499 (22%) in 2012. Integrase sequences were obtained from 67 individuals that failed on RAL during the study period. It represented 2.6% of RAL-treated patients. Overall 40.3% of this subset of patients harboured changes associated with RAL resistance.
The prevalence of DGV RAMs was as follows: L74M (1.5%), L101I (62.7%), E138K (1.5%), G140A (1.5%), G140S (10.4%), Q148H (10.4%), Q148R (1.5%), and G193E (1.5%). Changes at codon 148 were recognized in 8 individuals, all of whom also harboured 140 codon mutants. The pattern Q148H+G140S was present in 7 and Q148R+G140A in 1 patient.
The prevalence of EVG RAMs was as follows: Q148H (10.4%), Q148R (1.5%), N155H (23.9%), G140A (1.5%), G140S (10.4%), L74M (1.5%), T97A (6%), E138K (1.5%), Y143C (1.5%), Y143H (1.5%), Y143R (1.5%), and V151I (6%). Mutation N155H was found in 16 individuals (24%), of whom 14 also harboured L101I.
Using the 2013 version of the Spanish resistance interpretation algorithm, 8 genotypes (11.9%) from individuals that had failed on RAL should be considered as resistant to DGV, whereas 24 (35.8%) should be considered as EVG-resistant (p<0.001).

Conclusions: More than 20% of patients on antiretroviral therapy at our institution have been treated with RAL within the last five years, being failures recognized in <3% of them. Integrase resistance mutations were found in only 40% of this subset of patients, being N155H (23.9%) and Q148H (10.4%) the most common. Predicted DGV susceptibility was 90%, being down to less than 65% for EVG. This information may be valuable for the sequential use of integrase inhibitors.

No conflict of interest

Abstract: P_40

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

A complex role of structural proteins in the resistance of HIV-1 to PI

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Background: Various studies have shown that Gag or protease mutations both can affect HIV-1 fitness and protease inhibitor (PI) susceptibility through the need of a strictly ordered process of Gag/Gag-Pol polyprotein cleavage. Thus the forced co-evolution of protease and Gag as substrate helps explaining why various Gag cleavage site (CS) mutations can be linked to PI resistance. This study aimed at identifying new therapy- and resistance-associated mutational patterns in protease and/or Gag.

Materials and Methods: C-terminal subtype B Gag sequences including at least 10 codons flanking the p7/p1 and p1/p6 CS in combination with their corresponding protease sequences and complete treatment histories were obtained from routine diagnostics in the Basel and Zurich centers of the Swiss HIV Cohort Study. We

compared 264 PI treatment-naïve samples without primary resistance mutations ('TN+no_res') and 54 samples from PI treatment-experienced patients with viral loads ≥100c/mL carrying primary resistance mutations ('TE+res'). Fisher's exact test (alpha=0.05 as cut-off) was employed to identify differences in mutation frequencies between the groups and pairwise associations between selected mutations. We engaged suitable algorithms to identify signature patterns and extract mutagenetic trees.

Results: A significant overrepresentation of known therapy- and resistance-associated mutations in Gag (431V, 435E, 437V, 449V, 452S, 453A, 453L) for the TE+res group as well as primary and secondary resistance mutations in protease over TN+no_res group confirmed previously published information and served as validation for our method.
Beyond this we detected the novel mutations 429I (3.7% *vs* 0%; TE+res *vs* TN+no_res), 451H (3.7% *vs* 0%), 441Q (7.4% *vs* 1.1%), 427D (7.4% *vs* 0.4%) in Gag to be associated with the TE+res group. They had not previously been reported to be therapy- or resistance-associated.
Moreover, in the TE+res group mutation 427D was found to be associated with 54V, a primary resistance mutation in protease. We also found a new association for primary protease mutation with key position in Gag: 46I with 437I (wild-type, not therapy-associated). The 46I change was also negatively associated with 437V, which by itself is known to be resistance-related.
In the Swiss data set we identified patterns distinguishing TE+res from TN+no_res samples with a specificity of 98%, sensitivity of 65%. Unexpectedly wild-type at position 427T was critical for certain TE+res and certain TN+no_res defining patterns.
Mutagenetic trees were extracted and suggest that several distinct resistance pathways exist with conditioning first mutations to obtain patterns that included protease resistance mutations and Gag alterations.

Conclusions: Here we report novel mutations in Gag-Pol, which clearly associate with incomplete viral suppression in PI treatment-experienced patients, who carry HIV-1 with primary resistance mutations. We also identified new associations between these mutations and specific Gag mutations, which are therapy- or resistance-associated or with Gag positions that cannot be altered.

Our results invite the model that certain resistance-mutations in protease can favor or can be favored by a counterpart in Gag and that the role of a particular Gag mutation can greatly be affected by secondary alterations.

No conflict of interest

Abstract: P_41

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Proviral DNA as target of HIV-1 resistance analysis

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Background: HIV-1 resistance tests are routinely performed on the basis of viral RNA. As therapy failure according to the DAIG guidelines is defined with a viral load of >50 copies/ml and an accumulation of resistance mutations under failing therapy should be avoided, a change in medication in settings with low viral loads is required. As these viral loads are mostly too low to perform a successful resistance test with HIV RNA, the resistance analysis with proviral DNA provides a possible alternative.

Material & Methods: The plasma RNA and the corresponding proviral DNA of 80 EDTA samples of the RESINA cohort including 50 therapy naïve (TN) and 30 therapy experienced (TE) patients were isolated and analysed for their resistance profiles in the protease (PR) and reverse transcriptase (RT) genes.

Results: 78/80 samples (TN=48, TE=30) provided genotypes of viral RNA and proviral DNA.
In total we detected 9 samples with PR mutations und 30 samples with RT mutations. 23/39 samples (59%) with detected resistance mutations presented an equal or a higher number of mutations in proviral DNA compared to viral RNA, whereas the other 16 samples (41%) showed a lower number of mutations in proviral DNA.
NRTI mutations with 83.3% had the highest concordance of detected mutations in RNA and DNA. NNRTI mutations had a concordance of 69.9% and PIs of 80.8% (not yet statistically significant).
With respect to specific amino acid positions, the frequency of resistance mutations was higher in the viral RNA, except position 70 in the RT gene and position 30 in the PR gene. Mutations at these positions were detected more frequently in the proviral DNA.
Viruses of TE patients present more resistance mutations compared to viruses of TN patients, as expected. Samples of TE patients display comparatively more mutations in viral RNA, while TN patients archived more mutations in proviral DNA.

Conclusions: The genotyping of HIV proviral DNA provides useful information. The comparison of viral RNA and proviral DNA presents a higher detection rate of resistance mutations in proviral DNA in TN patients. In TE patients proviral DNA presented not the same quantity of information as the viral RNA, but it is helpful in cases of an unsuccessful resistance analysis with viral RNA and/or no existing historic resistance data. Therefore resistance analysis of proviral DNA can be supportive for the evaluation of resistance when viral load is low or not detectable in TE patients, in cases of unavailable historic resistance information and for detection of transmitted drug resistance in TN patients.

No conflict of interest

Abstract: P_42

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Low rate of antiretroviral resistance among treatment-experienced HIV-infected Romanian patients

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Introduction/Background: The combined antiretroviral therapy (cART) has led to a radical change in the evolution of HIV infection, including marked improvements in morbidity and mortality. However, the development of drug resistance, inadequate treatment adherence and suboptimal drug potency are important factors that may contribute to treatment failure. Our study evaluates the prevalence of virological failure and drug resistance in a group of HIV-infected Romanian patients with consistent, long-term, and well-documented cART treatment.

Materials & Methods: We studied a group of 164 long term survivors (median age 24 years), parenterally infected with HIV-1 during early childhood, with a median duration on HAART of 13 years and repeated virological failure. HIV viral load was tested by quantitative RT-PCR (Cobas TaqMan HIV-1 Test *Roche* Molecular Systems, *USA)*. Pol gene sequencing was performed using the ViroSeq HIV-1 Genotyping System (Abbott Laboratories, USA) for those with more than 1000 copies of HIV RNA/ml. HIV subtype was determined using the REGA HIV-1 subtyping tool; mutations associated with TDR were identified using the WHO 2009 list.

Results: Of 164 patients, 36.6 % are currently experiencing virological failure (HIV RNA >40 copies/ml). Determinants of virological failure included male sex, lower CD4 T-cell counts,

longer time on ART and longer exposure to monotherapy regimens. Only 18.5% of the patients (31/164) had plasma HIV-1 viral load ≥1,000 copies/ml. HIV-1 subtype analysis indicated that all strains belonged to the F subtype, which is most prevalent in Romania. 32.2% (10/31) of these treatment-failing patients remained susceptible to all ARV classes. 22.6% (7/31) presented resistance to two drug classes and 16.1% (5/31) had triple class resistance. Only 6.45% (2/31) of the subjects presented resistance to all currently available drugs. Mutations related to reverse-transcriptase inhibitors were predominant (NNRTIs: 48.38%, 15/31 patients; NRTIs: 41.93%, 13/31), followed by PIs resistance mutations (32.25%, 10/31). All the HIV isolates had multiple accessory resistance mutations at a high frequency in the PR and RT genes.

Conclusions: We report a low rate of ARV resistance associated with virological failure in a group of extensively-treated HIV-infected Romanian patients, long term survivors, parenterally infected in early childhood. One third of the patients with detectable HIV viral load had no resistance mutations suggesting adherence problems. The mutational pattern of HIV-1 reflects resistance to drugs that have been administered for long periods of time.

No conflict of interest

Abstract: P_43

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Inmuno-virological response to Darunavir versus Lopinavir containing ART regimens in HIV-2 infection

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Background: Lopinavir (LPV) and Darunavir (DRV) are amongst the most potent PI against HIV-2. Information on the resistance barrier and genotypic profile in HIV-2 patients failing on these drugs is scarce.

Methods: All HIV-2-infected patients included in the Spanish HIV-2 cohort who had received LPV or DRV were examined. Virological response was defined as achievement of <50 HIV-2 RNA copies/mL. Immunological response was defined as achievement of CD4 counts >200 cells/mm^3. Population sequencing was used for characterising genotypic resistance mutations in DRV and LPV failures.

Results: From a total of 289 HIV-2 patients recorded up to October 2013, 38 had received LPV and 24 DRV. Main features: 37 (60%) male, median age 43-years-old (range 26-67); country of origin: Sub-Saharan Africa 47 (78%); native Spaniards 8 (13%); Portugal 4 (7%) and Russia 1 (1%). Heterosexual transmission 48/62. Most common nucleoside backbone taken along with LPV or DRV were Truvada (n=29; 47%), Kivexa (n=5; 8%), and Combivir (n=13; 21%). At baseline, median CD4 count was 210 [136-400] cell/mm^3 and median HIV-2 RNA was 1.69 [1.69-2.6] log cop/mL. Patients who initiated DRV had greater viral load and lower CD4 counts than those who begun LPV: 2.11 vs 1.69 (p=0.05) log HIV-2 RNA cop/mL and 199 vs 218 cells/mm^3; p=0.24).
Most patients achieved undetectable viremia using either LPV (90%) or DRV (80%) (p=0.42). Moreover, immunological response occurred in 76% of LPV and 66% of DRV patients (p=0.73). At the end of follow-up, median CD4 counts were 320 on LPV and 288 cells/mm^3 on DRV (p=0.44).
Finally, resistance mutations were found in 8/24 DRV failures versus 5/38 LPV failures (p=0.05). Resistance mutations selected on DRV failures were: I54M (5); I54L (1); I84V (5); I50V (1); V71I (1); I82F (1); and L90M (1).
Resistance changes in LPV failures were V47A (2); I50V (1); I54M (1); V62A (1); I82F/L (2); I84V (2); and L90M (1).

Conclusions: Both LPV and DRV seem to be similarly effective in HIV-2 patients. However, DRV might depict a lower resistance barrier than LPV.

No conflict of interest

Abstract: P_44

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Phenotypic characterization of a unique HIV-1 strain with a two-aminoacid deletion in the ß3-ß4 hairpin loop domain of reverse transcriptase

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Introduction/Background: Single deletions of codons 67, 68, 69 or 70 in the β3-β4 hairpin loop domain of HIV-1 reverse transcriptase (RT) are rarely selected in vivo (<0.2% of treated patients), mainly after exposure to nucleoside or nucleotide inhibitors (NRTI). Depending on background mutations, these single deletions modify the RT structure with hardly predictable consequences on the susceptibility to nucleoside analogs, thus decreasing the likelihood of correct drug resistance prediction with genotypic algorithms. Here we report a phenotypic characterization of a unique HIV-1 strain carrying a two-aminoacid deletion (codons 68-69 of RT) together with M41L, A62V, M184V and K219R, identified in a patient under NRTI based therapy.

Materials & Methods: The viral strain with the double deletion at codon 68-69 was identified during drug resistance monitoring in a patient under failure of an antiretroviral therapy comprising saquinavir, lamivudine and stavudine. After the amplification of the entire RT and RNAseH region from viral RNA, the PCR fragment was used to produce a chimeric virus through homologous recombination with a modified version of pNL4-3 vector in 293FT cells. The recombinant virus was employed to infect a reporter cell line (TZM-bl cells) in presence of 5-fold dilutions of lamivudine, stavudine, zidovudine, abacavir, tenofovir and the IC_{50} values were compared to those obtained with the NL4-3 virus. The prediction of NRTI susceptibility of the codons 68/69-deleted strain was analyzed by the HIVdb, Rega and ANRS algorithms through the HIValg tool available at the Stanford HIV Drug Resistance Database web site.

Results: Phenotypic evaluation revealed that the recombinant virus carrying patient derived RT sequence with deletion of codons 68-69 was highly resistant to 3TC (fold change [FC] >1000) and showed reduced susceptibility to stavudine (FC 1.8) and abacavir (FC 1.8). By contrast tenofovir and zidovudine had fold change values suggesting possible hypersusceptibility (0.85 and 0.35, respectively). With the exception of lamivudine and emtricitabine, the three algorithms showed several differences in predicted susceptibility levels to one or more NRTI. The phenotypic hypersusceptibility to zidovudine was in contrast with the intermediate resistance call by HIVdb and REGA while ANRS predicted susceptibility to this drug. Intermediate resistance to tenofovir was also called by HIVdb, as opposed to susceptibility by ANRS and REGA.

Conclusions: This is the first phenotypic characterization of a unique viral strain with a 2-aminoacid deletion in the RT β3-β4 domain. The observed zidovudine hypersusceptibility was previously reported with some 1-aminoacid deletion mutants. Genotypic interpretation systems were mostly discordant among each other, with ANRS calling susceptibility with most NRTI as opposed to HIVdb and REGA yielding more cautious predictions. This case study confirms the essential role of phenotypic investigation as a diagnostic tool for the optimization of antiretroviral therapy in the presence of unusual HIV-1 strains.

No conflict of interest

Abstract: P_45

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Predicted efficacy of Dolutegravir in an HIV cohort of patients failing a Raltegravir based regimen

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Background & Aim: Dolutegravir (DTG) is a second-generation integrase inhibitor (INSTI) with a higher genetic barrier to resistance than first generation INSTIs. In this study we have analyzed the predicted efficacy of DTG in patients who have previously failed a Raltegravir (RTG) containing regimen.

Patients and methods: HIV Integrase was sequenced by means of an 'in house' protocol and later using the Trugene HIV-1 platform (Siemens). To evaluate resistance to DTG we have used the Stanford HIV database, and we have also used a recent clinical outcome based algorithm (Vavro C, Intl HIV & Hepatitis Workshop, 2013), that considers intermediate resistance when Q148HKR and one of L74I, E138AKT, or G140ACS are present, and fully resistant if Q148HKR and two or more of L74I, E138AKT or G140ACS are detected.

Results: We have examined 77 RTG failing patients, 68.8 % men, with a median age of 46.8 years old [IQR: 41.1-51.6], median viral load of 3.5 logs c/ml [2.5-4.8], median CD4 of 244 cells/ml [101-460]. Raltegravir was in combination with one or more NRTI and a protease inhibitor (PI) in 30.3% of the cases, and with maraviroc and a NRTI or a NNRTIs or a PI in 22.4% of the patients. INSTI mutations were detected in 22.1% of the patients, being N155H (13%) the most prevalent mutation, followed by T97A (7.8%), L74I (3.8%), Q148H (2.6%) and G140S, T66I, Y143C (1.3% each), being 18.2% of the patients with any degree of resistance to RTG following Stanford interpretation. As for DTG, we found four cases with intermediate resistance (Q148H+G140S, n=1; Q148H n=1; N155H+T97A; n=2) following the Stanford interpretation. According to the Vavro algorithm, we found only one case (1.3%) with intermediate resistance to DTG (Q148H + G140S).

Conclusion: Dolutegravir resistance in patients failing a Raltegravir containing regimen in our cohort is rare, making this drug an excellent alternative for salvage in patients who fail to this first generation integrase inhibitor.

No conflict of interest

Abstract: P_46

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Algorithms evolution in drug resistance interpretation: ANRS, REGA and Stanford HIVdb

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Background: HIV-1 genotyping has become a widely accepted tool for monitoring antiretroviral (ARV) therapy. Various genotypic resistance interpretation tools have been developed in recent years. Previous comparative assessments of these algorithms showed some variability. The ANRS, REGA and Stanford HIVdb are three commonly used and publicly available drug resistance interpretation systems. The aim of this study was to compare them side-by-side and over time.

Methodology: ANRS AC11 2004, REGA 6.2 and HIVdb 3.9 (2004), or ANRS AC11 2012, REGA 8.0.2, HIVdb 6.2.0 (2013) were used to compare the ARV-drug status for each sequence. Each algorithm has different classifications for drug susceptibility. In this study, we used a simplified three-category classification scheme: sensitive, intermediate, and resistant. Results were considered as discordant when one algorithm assigned the

level 'resistant' whereas another assigned 'sensitive'.

Results: Two hundred eighty four EDTA-plasma samples were included in the over time study (clinical samples from 2002 to 2004). In 2004, discrepant results were found in 24% of the samples; discrepancies were more often associated with Nucleoside Reverse-Transcriptase Inhibitors (NRTIs) (4.4%) than with Non-Nucleoside Reverse-Transcriptase Inhibitors (NNRTIs) (1.9%) or Protease Inhibitors (PIs) (1.7%). When the same data set was reanalyzed in 2013, discordant results were observed for 26% of the samples. Discrepancies were more often associated with NRTIs (3.2%) than with NNRTIs (2.6%) or PIs (2.2%). Despite the same general distribution, the observed discrepancies change over time and do not relate to the same drugs in 2004 and 2013. This reflects the integration of new drug resistance information but also the difficulty to harmonize the mass of information.
Four hundred forty six EDTA-plasma samples were included for the second study period from 2010 to 2013. Discrepant results were found in 49% of the samples. Discrepancies were more often associated with PIs (5.8%) than with NNRTIs (2.5%) or NRTIs (1%). Interestingly, most discrepancies could be attributed to analyzing the samples using the ANRS algorithm. The distribution of discrepancies between drug classes is not the same as in the over time set. This could be explained by the introduction of new ARV drugs and thus the selection of relevant resistance mutations.

Conclusions: As previous observations, our study demonstrated that the three most common used interpretation systems differ in their overall prediction of drug resistance to cART. This can be explained by: (i) the fact that multiple mutations are required to confer resistance to PI and NRTI resulting in complex mutational patterns more difficult to interpret; (ii) as the three evaluated algorithms use different interpretation models, discordances for complex patterns are inevitable. Thus, this study has implications for clinicians, who, faced with complex genotypic interpretation, should realize that all genotypic interpretation algorithms may not provide the same resistance interpretation. This may be of particular importance when patients move between regions, in which resistance testing uses different systems.
In conclusion, our study demonstrates that even after many years of genotypic resistance interpretation further adjustments and improvements in the interpretation tools continue to be needed.

No conflict of interest

Abstract: P_47

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Epidemiology of transmitted drug resistance in newly diagnosed HIV-1 individuals in Malaga (Spain) from 2004 to 2013

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Background: Transmitted drug resistance rates among newly diagnosed individuals vary widely in different regions of the world depending on the characteristics of the study population, HIV subtype, major risk factors, period of time that carries out the study, access to drugs and the interpretation of susceptibility. The purpose of the present study was to describe the trend and epidemiology of transmitted drug resistance and HIV subtype among newly diagnosed treatment naive HIV-1 patients diagnosed in Malaga (Spain) from 2004 to 2013

Material and methods: Virus from 606 individuals diagnosed with HIV-1 in Malaga between 2004and 2103 was analyzed for transmitted drug resistance and HIV subtype. The PR and RT coding regions of the pol gene were amplified and sequenced using the TRUGENE HIV-1 genotyping kit (Siemens). Drug resistant mutations were interpreted following the latest IAS_USA panel list and the Spanish HIV Research Network algorithm (RIS). Genetic subtypes were identified using Geno2pheno bioinformatics tools. Information about age, gender, origin, transmission route

and prior negative HIV test were available from questionnaires from the study. We distinguished three periods: A(June 2004-June 2007), B (July 2007-December 2010) and C (January 2011-December2013)

Results: Of the 606 patients, 206 were diagnosed in Period A, 193 in Period B, and 207 in Period C. Overall, 86,8% were male, with median age of 36 years, median baseline CD4count 355 cells/ml and median baseline viral load of 4,7 log10. 18% were seroconverters and non B subtypes were recognised in 16,9%.Although 10 different subtypes were identified, subtype B infections predominated (83,1%). 71% of the patients included were born in Spain, and 67,3% reported route of infection as homosexual transmission. Rates of transmitted drug resistance were 20,8%, 22,2%and 28,9% in each period. In NRTI, the most common primary mutations were D67N,T69D/N, M184V, T215S/L and K219E/Q. In NNRTI: K103N, V106A, V179D and G190A/S. In PI the most prevalent mutation was M46I/L. We identified some transmitted multidrug resistant HIV among the patients.

Conclusion: The transmission of drug resistance mutations and non B subtypes is stabile in our area. The prevalence of some mutations suggests possible transmission chains. This result underlines the significance of baseline resistance testing in newly diagnosed HIV-1 individuals

No conflict of interest

Abstract: P_48

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Detection of HIV-1 drug resistance mutations by bulk population sequencing assay and Next Generation GS Junior System

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Background: Treatment guidelines recommend testing for HIV-1 drug resistance by bulk population sequencing before starting antiretroviral drugs or adapting subsequent regimens. Traditional sequencing assays detects only variants present in at least 15-25% of the entire virus population. Several studies have shown it could be clinically relevant to drop below this detection cut-off. Here we analyzed IAS and non IAS defined NRTIs, NNRTIs, PI drug resistance mutations detected by Next Generation GS Junior System and compared it with a bulk population system routinely used in clinical practice.

Materials & Methods: Next generation sequencing (NGS) was performed starting from plasma samples of 69 infected individuals, collected through routine HIV-1 drug-resistance testing by Viroseq HIV-1 Genotyping System (Abbott Molecular). Amplicon Sequencing of HIV RT and PR Plate (Roche Applied Science) was performed following the manufacturer's instructions. HIV-1 variants was analyzed by a specific HIV-1 tool by AVA software vs. 2.7. Updated International AIDS Society (IAS) drug-resistance mutations list (March 2013) was considered for the analysis of resistance positions.

Results: The median HIV-RNA of the plasma samples included in the study was 4,6 log copies/mL (range 2,6-6,5). 459 out of 561 IAS defined variant positions were identified. The bulk population sequencing did not detect any mutations below a 10% prevalence. The performance of the test was 20% for a prevalence between 10 and 15% and increased to 57%, 75% and 85% for prevalence 15-20%; 20-25% and 25-30%. Above the 30% threshold the detection was 100%. Overall 38% of IAS defined mutations (177 mutant codons) were detected by the Next Generation GS Junior System below the 10% prevalence limit. Among these several clinically relevant reverse transcriptase mutations were included: K65R (1 case); D67N (2); K70R (2); F77L (2); K101E (2); V108I (6); E138A (2); E138K (3); E138Q (1); Y181C (1); M184I (1); M184V (3); Y188C (1); Y188H (4); K219E (1); K219Q (1) and H221Y (1). Only one major protease mutation was observed D30N (1 case). In the grey zone between 10 and 30% prevalence 23 mutations were present (5% of total) and included some

clinically relevant mutations such as reverse transcriptase M184I; T215Y; E138A. There was no significant difference between IAS and non-IAS defined mutations, being the prevalence in the circulating virus the only limiting factor.

Conclusions: Next Generation GS Junior System using HIV Plate detected low-frequency HIV-1 variants harboring mainly RT drug resistance mutations that could affect the antiviral therapy outcome. NGS may be more useful than bulk population sequencing to detect resistant variants. The routinely use requires standardized and validated protocols.

No conflict of interest

Abstract: P_49

Novel Diagnostic Technologies & Approaches

Comparative analysis of Hepatitis C virus genotyping methods for discriminating rare subtypes in Venezuela

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Introduction: Infection by hepatitis C virus (HCV) is a public health problem and represents a major cause of chronic liver disease worldwide. This virus has an RNA genome and displays a high degree of genetic variability, classified in 7 genotypes and numerous subtypes. Genotype determination is important from a clinical point of view, for the selection of an appropriate antiviral treatment, as well as the dose and duration of it. In Venezuela, sequence analysis of the NS5B region allowed classifying the most common subtypes in the country as 1a, 1b and 2j.Due to the abundance of a rare subtype (G2j) and the presence of other minor subtypes of G2 in the country, the aim of this study was to evaluate the performance of common genotyping tests to discriminate this subtype.

Materials and methods: A total of 67 samples from individuals with HCV infection were analyzed, prior informed consent and approval of the Bioethics Committee of the National Institute of Hygiene "Rafael Rangel". HCV genotyping tests used were reverse line blot hybridization (Versant HCV Genotype 2.0 LiPA) and sequencing of the 5 'NC and NS5B regions of HCV (5NCS and NS5S).The degree of agreement between tests was carried out by calculating the kappa coefficient, an instrument designed to adjust the effect of chance in the proportion of the observed concordance. A dendogram performed according to the band presence and intensity in the LiPA test was compared to the homology tress obtained from sequence analysis.

Results: The determination of genotype and subtype, taking as reference test the NS5S sequencing, showed a high degree of concordance (100% and 98%), in the genotype assignment, and 67% and 59%, in subtype assignment, when evaluating 5'NCSand reverse line blot hybridization, respectively. Discrepancies at subtype level were observed in 17 and 21 samples by 5'NCS and reverse line blot hybridization, respectively. The NS5S allowed for the identification of subtypes 2j and 2s, not discriminated by 5'NCS. However, PCR was more sensitive to amplify 5 'NC region (96% compared to LIPA), vs. 78% of for NS5B region PCR amplification. No specific band pattern was observed in LIPA for G2j isolates, and no good correlations were observed when comparing LIPA dendograms for each genotype with homology trees neither for 5NCS nor for NS5S.

Conclusions: The correlation between the studied methodologies, with respect to the reference test was: “good” for 5NCS and “moderate” for LIPA. The analysis of the HCV 5'NC region can lead to mistakes at the level of subtypes, so the presence of some subtypes may be underestimated, as is the case G2j and other G2 subtypes in our country.

No conflict of interest

Abstract: P_50

Novel Diagnostic Technologies & Approaches

Clinical impact of Ultra-Deep versus Sanger Sequencing detection of minority mutations on the HIV Drug Resistance genotype interpretations after virological failure

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Background: Drug resistance mutations are routinely detected using standard Sanger sequencing, which does not detect minor variants with a frequency below 20%. The impact of detecting minor variants generated by ultra-deep sequencing (UDS) on HIV drug-resistance (DR) interpretations has not yet been studied.

Material & Methods: Fifty HIV-1 patients who experienced virological failure were included in this retrospective study. The HIV-1 UDS protocol was performed using the GS Junior (Roche 454 Life Sciences Branford, CT). This UDS protocol allowed the detection and quantification of minor and major HIV-1 protease and reverse transcriptase variants related to genotypes A, B, C, E, F and G. DeepChek®-HIV (ABL, SA and TherapyEdgeTM, USA) simplified DR interpretation software was used to compare Sanger sequencing and UDS at two different thresholds (≥1% and ≥20%). DeepChek®-HIV utilises the ANRS, HIVdb and Rega algorithms.

Results: The total time required for the UDS protocol was found to be approximately three times longer than Sanger sequencing with equivalent reagent costs. UDS detected all of the mutations found by population sequencing and identified additional resistance variants in all patients, primarily by using 1% sensitivity. An analysis of DR revealed a total of 643 and 224 clinically relevant mutations by UDS and Sanger sequencing, respectively. Three resistance mutations with >20% prevalence were detected solely by UDS: A98S (23%), E138A (21%) and V179I (25%). A significant difference in the DR interpretations for 19 antiretroviral drugs was observed between the UDS and Sanger sequencing methods. Y181C and T215Y were the most frequent mutations associated with interpretation differences. The major discrepancies between Sanger and UDS were primarily found at the 1% threshold in the three algorithms.

Conclusions: UDS was more sensitive than the standard Sanger sequencing. A combination of UDS and DeepChek® software for the interpretation of DR results saved a considerable amount of time and would help clinicians provide suitable treatments. A cut-off of 1% allowed a better characterisation of the viral population by identifying additional resistance mutations and improving the DR interpretation.

No conflict of interest

Abstract: P_51

Novel Diagnostic Technologies & Approaches

Development of a method for DBS/DPS HIV-1 genotypic tropism determination

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Background: Maraviroc (MVC) an entry inhibitor, binds to the CCR5 co-receptor inhibiting replication of CCR5 using (R5-tropic) HIV-1 strains. As MVC has no antiretroviral effect on strains using the CXCR4 co-receptor (X4-tropic), determination of co-receptor usage (viral tropism testing) is needed to exclude the presence of X4-tropic viruses. For

determination of viral tropism several phenotypic and genotypic assays have been developed. Genotypic tropism tests, analyze the sequence of the HIV- 1 envelope gp120 variable 3 (V3) loop, the main determinant for co-receptor usage. The objective of this work was to develop a field friendly method for collection, transport and storage of samples obtained to determine genotypic viral tropism, using Dried Blood Spots (DBS) and Dried Plasma Spots (DPS).

Methods: From November 2013 to January 2014 we collected 3 different samples from 6 HIV-1+ patients, 1 ml plasma, DBS and DPS. For the DBS we spotted 200ul of whole blood and for the DPS 200ul of plasma separated through gravity in S&S 903 paper. Spots were store at room temperature in a zip-lock bag. RNA was extracted from DBS and DPS samples with QiaAmp Viral RNA Mini Kit Qiagen after 48hrs and 1 week of sampling. Plasma was frozen and RNA extracted 48 hrs. after sampling. RNA was amplified using a RT-PCR and a Nested PCR using primers published by the ANRS Group, and sequenced. Tropism was determined in each sequence using the Geno2Pheno algorithm with a 10% FPR.

Results: Genotypic tropism from the six cases was determined as 4 R5 and 2 X4. 14 samples of DBS and DPS were sequenced and all had the same tropic strains as the plasma from the same individual. We didn't find any discrepancy in the tropism result between plasma, DBS and DPS at any time. DBS: 5 samples of DBS extracted at 48 hrs were determined as R5(3) or X4 (2) and 2 samples extracted at 1 week (1 R5 and 1 X4). DPS: 4 samples of DBS extracted at 48 hrs were determined as R5(2) or X4 (2) and 3 samples extracted at 1 week (2 R5 and 1 X4). Independently of DBS or DPS samples and time of extraction FPR values were equal in 5 of the 6 comparisons for X4 samples and the difference in the sixth sample was only 0.4%. On the other hand FPR values in R5 samples varied from 0 to 7.2% without relation to type of sample or time of extraction.

Conclusions: DBS and DPS samples with the developed method were equally valuable for the determination of genotypic tropism from HIV-1-infected individuals. Correlation is complete with referral plasma with preservation of the spots for 48 hrs or one week. The use of DBS/DPS for HIV-1 genotypic tropism assays, could optimize collection, transport and conservation of samples from distant places.

No conflict of interest

Abstract: P_52

Novel Diagnostic Technologies & Approaches

Hepatitis C virus high resolution subtyping, variant calling and drug resistance determination from Next Generation Sequencing (NGS) data

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Background: Compared to Sanger sequencing, NGS provides the ability to detect mutations present at low frequencies, some of which are potentially leading antiviral treatment failure. Yet, NGS data comes at the cost of increased complexity. Additionally, as the number of new direct antiviral agents (DAAs) approved for the management of chronic HCV infection increases, reliable analysis software that can use NGS data and be frequently updated with the most recent drug resistance information is in demand. We developed DeepChek-HCV, a sequencing analysis system for HCV.

Methods: We used DeepChek-HCV, a secured application following good practices for software development, to perform within minutes high-resolution subtyping and HCV resistance interpretation of NGS data from 15 Roche 454 runs containing 2 amplicons overlapping the 5'UTR and NS5B regions. Results and user-friendliness were compared to a pipeline developed by the Vall d'Hebron Institut of Research (VHIR-HUVH). Drug resistance was determined at different sensitivity levels (down to 1%) using 3 different drug resistance guidelines (Geno2Pheno, IAS

and literature review) for the main DAAs including Boceprevir, Telaprevir, Sofosbuvir, Daclatasvir.

Results: Our panel of 370 samples contained 58141 unique haplotypes. DeepChek-HCV took ~8 minutes to classify all haplotypes while VHIR-HUVH identified a subset of 672 haplotypes (representing 48.8% of the reads) in ~4.5 hours. In the 672 sequences subtyped by VHIR-HUVH, 97.3% matched the subtype determined by DeepChek-HCV. The 18 cases with discordant results were typically associated with a low confidence levels (<60%) in at least one of the algorithms, and most were related to genotype 2 and/or to the 5'UTR region, which is less discriminative than NS5B. Clinical reports containing the genotyping and drug resistance assessments were produced for validation by a panel of experts who confirmed its usefulness.

Conclusions: This study illustrates the benefits of using well-validated downstream companion diagnostic software combining high-resolution subtyping and drug resistance assessment of major and minor variants, for the management and profiling of HCV patients in order to target personalized medicine.

Conflict of interest Employee and shareholder of ABL

Abstract: P_53

Novel Diagnostic Technologies & Approaches

Use of reverse transcriptase and protease massive paralell sequencing to investigate viral diversity and HIV microvirome.

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Background: It has been postulated that in early stages of HIV infection a single strain, considered as the founder virus, finally establishes infection, and is the parental one of all other that arise under immune and pharmacological pressure. Recently there has been interest in the characterization of founder viruses, mainly as a target to study neutralizing antibodies for vaccine development.

Objective: to study if reverse transcriptase and protease ultradeep pyrosequencing (UDPS) may help to identify the degree of variability in the different quasispecies present in HIV infected patients.

Patients and Methods: A total of 12 plasma samples from HIV primoinfected patients [contact risk (12/12) and/or p24Ab seroconversion (5/12) and/or presence of p24Ag (9/12) and/or previous HIV-negative serology (3/12)] and from 24 naïve patients of unknown duration have been studied. Four regions of the *pol* gene [protease (RTP-A: 183-465nt) and three fragments of RT (RTP-B: 414-714nt, RTP-C: 623-939nt, RTP-D: 897-1206nt] were amplified and subsequently quantified and purified to sequence a pool of 2 * 10e6 molecules in a 454 GS Junior. Finally, the sequences were analyzed using Amplicon Variant Analyzer (AVA), and variability was measured by calculating the percentage of the single majority sequence.

Results: A median of 9007 sequences per patient was obtained (IQR= 6104-10167). The median viral load for naives was 109644 c/ml and 1.69 million c/ml for primoinfected. For naïve patients a single majority sequence was present as 21.6% for RTP-A (RIQ= 16.4-37.2), 28% for RTP-B (10.74-35.5), 22% for RTP-C (11,4-34,6), and 26% for RTP-D (11,59-40,18). On the other hand in primoinfected patients, a single majority sequence was found in 80% for RTP-A (73-85,47), 82.5% for RTP-B (71,7-86,66), 72.4% for RTP-C (65,01-80,53) and 75.4% for RTP-D (68,9-78,83). Significant differences were found between groups for all the analysed fragments (p<0.0001)

Conclusions: Ultra deep pyrosequencing of reverse transcriptase and protease allows to know the degree of variability in HIV infected patients, and is a support to identify primary infection, allowing to study the HIV microvirome in a single patient.

No conflict of interest

Abstract: P_54

Novel Diagnostic Technologies & Approaches

Improving Sanger and Next Generation Sequencing (NGS) HIV data management using downstream analysis systems tailored for clinical diagnostics or research applications

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Background: Both Next Generation Sequencing (NGS) technologies and Sanger sequencing are currently in use for HIV drug resistance genotyping. The complexity of the generated data, particularly in the case of NGS, requires advanced diagnostic systems capable of being frequently updated with the most recent drug resistance information. Here we describe DeepChek®-HIV (DC) and ViroScore®-HIV (VS), the first CE-IVD marked medical devices for NGS and Sanger, respectively.

Methods: The DC and VS systems were designed to perform automated analysis and HIV resistance interpretation of both Sanger sequencing data (DC and VS) and NGS aligned sequences (DC) all in a matter of minutes. DC accepts alignment files in either standard BAM format or FASTA file format produced by AVA (454 - Roche) and also sequence files with unaligned reads for high resolution subtyping only. Several clinical guidelines for drug resistance assessments, including HIVDB, ANRS and Rega, are made available to the user giving the ability to compare algorithms. Many aspects of the analysis and reporting can be customized creating user profiles to assure the production of consistent reports over time. Specific bioinformatics improvements were developed and quality controlled using VisibleChek®, a data mining application, to address the major issues influencing treatment decision (insertions, deletions and homopolymer corrections). Both DC and VS are secured applications following good practices for software development and were validated for safety and efficacy in several research and diagnostic laboratories.

Results: We analysed in 6 months 3300 samples from various cohorts worldwide with both DC and VS. First we isolated baseline patients from some cohorts. DC identified over 40% new variants missed by Sanger sequencing while also detecting the majority (94%) of the substitutions identified by Sanger. The variant calling component of the algorithm demonstrated both accurate classification of low-frequency variants generated by the NGS-454 GS-Junior platform and the efficient handling of homopolymer sequencing errors. Thus, a new insertion/deletion correction was developed for the detection of amino-acid insertion and deletion events of variable length. We tested this feature in a specific panel of 170 samples where DC identified new insertions (prevalence >=1%), in 7% of the samples all of which missed by alternative technologies. Other features were included to enhance the clinical genotyping report (predictive GSS determination and calculation of risk of virologic failure in drug-resistant minority variants).

Conclusions: This evaluation, part of the post-marketing surveillance and vigilance system, improved the variant validation expert system with additional controls. DeepChek®-HIV and ViroScore®-HIV are CE-IVD marked downstream analysis platforms for HIV genotyping and drug resistance testing. It optimally managed the analysis and comparison of NGS and Sanger data. It should provide additional reliable guidance to ARV treatment choices.

Conflict of interest: Employee and shareholder of ABL SA

Abstract: P_55

Novel Diagnostic Technologies & Approaches

Improved HIV-1 drug-exposure reconstruction with linear support vector machines

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Background: Reconstruction of an HIV strain's drug exposure can be useful where a patient requires a new treatment and treatment history is unavailable. For this purpose, drug-resistance mutations can be used. In this study, we assess the additional benefit of using support vector machines (SVMs) when reconstructing drug-exposure from genotype.

Materials & Methods: A dataset of 64,779 HIV-1 Pol amino-acid sequences was compiled from 16,242 therapy-experienced sequences with complete treatment history and 14,250 therapy-naïve sequences from the EuResist database. These were complemented with 34,287 therapy-naïve sequences from the Los Alamos National Laboratory Sequence Database, forming the *pooled dataset*. A total of 6,478 sequences (approximately 10%) were uniformly drawn from the pooled dataset to form *test set A*. Further 1,517 therapy-experienced sequences with complete treatment history were downloaded from the HIVdb TCE repository and assigned to an independent *test set B*. Training datasets of maximal size were drawn from the pooled dataset excluding test set A containing equal numbers of sequence instances exposed and not exposed to the drug in question, respectively. For each drug in a training dataset, a linear SVM was trained to discriminate between sequences that have been exposed to the drug and those that have not been exposed to the drug. Additionally, an SVM was trained to discriminate between therapy-naïve and treated sequences. Performance was assessed in terms of area under the ROC curve with 10-fold cross validation on both test sets. For comparison, both test sets were interpreted with the systems ANRS 09/2012, HIVdb 6.0.6, HIV-GRADE 06/2013, and REGA 8.0.2. For ROC curve calculation, susceptible-intermediate-resistant (SIR) predictions were assigned the following values: 0 for susceptible, 1 for intermediate and 2 for resistant. For prediction of therapy-naïve sequences, negative SIR values were added for all drugs tested.

Results: Cross-validation performance ranged from 0.72 to 0.90 with an average AUC of 0.81. Performance on test set A ranged from 0.61 to 0.90 with an average AUC of 0.77. Performance on test set B ranged from 0.58 to 0.83 with an average AUC of 0.71. When using test set A and comparing the performance of the linear SVMs to the four aforementioned interpretation systems, the linear SVM presented the best drug-exposure prediction performance for 14 out of 16 tested drugs and for the prediction of therapy-naïve sequences. On test set B, the linear SVM presented the best drug-exposure prediction performance on 10 out of 16 tested drugs; for two drugs, performance was worst. Linear weights were extracted from the SVMs, elucidating the importance of each amino-acid mutation in sequence classification. The largest weights driving the classification towards exposure to a certain drug coincide with established drug-resistance mutations for the drug in question.

Conclusions: The linear-SVM approach presented a good performance in predicting whether an HIV-1 Pol amino-acid sequence has been exposed to a certain drug. For the majority of drugs tested, prediction performance was superior to that of popular rules-based interpretation systems. SVM performance superiority could stand the test with an independent set.

No conflict of interest

Abstract: P_56

Novel Diagnostic Technologies & Approaches

A Next Generation Sequencing Assay for HCV Genotyping and Direct Antiviral Resistance Detection

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Background: Reliable determination of the hepatitis C virus (HCV) genotype/subtype is required to establish the choice and duration of antiviral treatment. A major issue that limits the efficacy of DAAs, in particular for individuals infected with the difficult to treat genotype 1, is the selection of resistance variants upon drug administration. Here we demonstrate a joint genotyping and resistance variant sequencing assay, and compare genotypic results of the DxSeq assay (Pathogenica, Inc.) with the Trugene HCV 5'NC genotyping assay (Siemens).

Methods: To allow detection of HCV genotypes 1-6 and resistance variants within the viral population of a patient sample, the DxSeq assay uses a multiplexed library of 436 HCV DxSeq probes. These probes target known resistance loci within the HCV NS3, NS5a and NS5b genes. DxSeq probes are sequencer-agnostic and can be used on all commercially available sequencing platforms. The assay is performed in a single tube, in under 15 hours on the Ion Torrent PGM, and provides significant read depth (500 to >50,000 reads per sample), which can be used to determine quantitative variant information. DxSeq libraries prepared from clinical samples were sequenced using an Ion Torrent PGM and analyzed by proprietary automated software analysis tools.

Results: We tested DxSeq in 21 DAA naïve HCV infected patients (13 HIV coinfected). Patient's median age was 45.5 [IQR 41.4-51.3], 66.7% were males, median HCV viral load (log) was 6.5 IU/ml [IQR 6.2-7.0]; for coinfected patients, median CD4 count was 237 cells/µl [IQR 81.5-497], median HIV VL (log) was 4.7 copies/ml [IQR 2.2-5.6], and 5 patients had a VL<20. Compared to the DxSeq assay, 6 samples (28.6%) were misclassified by the Trugene HCV 5'NC genotyping assay. Of these, 3 DxSeq genotype 1 samples had been incorrectly identified as genotype 4a by Trugene (two subtype 1a and one subtype 1b), and three 1a samples had been misclassified as subtype 1b. DxSeq probes additionally reported nucleotide and amino acid variants that confer drug resistance in NS3, NS5a and NS5b proteins. Minority variants were detected with a sensitivity of down to 1%, including amino acid polymorphisms V36I, Q80K, and E176G in NS3; Q30R, L31M, and P58S in NS5a; and M71V, C316S/Y, and V499A in NS5b.

Conclusions: HCV DxSeq identifies and corrects errors due to the Trugene HCV 5'NC assay in determining geno-/subtypes of clinical samples. Moreover, DxSeq probes allow for simultaneous detection of sequence variants associated with resistance to antiviral drugs such as boceprevir, telaprevir, simeprevir, daclatasvir.

No conflict of interest

Abstract: P_57

Novel Diagnostic Technologies & Approaches

Drug concentrations of Maraviroc, Darunavir and Ritonavir as a dual therapy switch in virologically suppressed patients (multicenter study GUSTA)

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Background: The GUSTA trial is designed to demonstrate the non-inferiority of therapeutic switch to maraviroc(MVC)+darunavir/ritonavir(DRV/r) 300/800/100 mg QD against the continuation of previous triple cART in patients with stable virological suppression. Recently a reduced exposure to MVC was observed in patients on DRV/r as compared to other boosted PIs, moreover a large randomized study of MVC+DRV/r 150/800/100 mg in naïve patients has been halted due to inferiority. We aimed at measuring drug levels and relating them to therapeutic outcome in GUSTA

Materials & Methods: GUSTA enrolls patients on antiretroviral therapy with HIV1-RNA<50 copies/µl>6 months, R5 tropism, CD4>200 cells/µl>3 months. The viral tropism is predicted using a single sequence of the V3 region of viral DNA from whole blood (geno2pheno,10%FPR). The plasma samples were collected at week 4, 12, 24, 36, 48, 60, 72, 84, 96 after switch. Maraviroc, darunavir and ritonavir levels were analyzed in the study arm in all plasma samples available at all time points using an UPLC-MS/MS method. Samples collected between >12-27 hours after maraviroc intake and >18-27 hours after darunavir intake were used for estimating Ctrough for these drugs. Spearman test was used to investigate bivariate correlation among drug concentrations. Linear and logistic regressions were used to analyze drug concentrations correlates.

Results: We included 18 patients for Ctrough analysis: 77.8%(14/18) were males, 89%(16/18) caucasian, 50%(9/18) heterosexuals, median age was 36 years (IQR 31-45), CD4 682 cell/µl (489-722), time from HIV diagnosis and duration of previous ART 14 (5.85-16.2) and 9.1 (5.4-15.5) years, respectively. The last regimen prior to switch included boostedPI in 83.3%(15/18), NNRTI in 5.6%(1/18), INI in 11.2%(2/18). Median self-reported adherence rate was 90% (82.5-90). In 78 follow-up samples from 18 subjects HIV-RNA was detectable at<50 copies/ml in 11.7% (15/78), >50 cp/ml in 3.1% (4/78), without any protocol-defined virological failure (1 HIVRNA >1000 cp/ml or 2 >50 cp/ml). The median Ctroughs were: for darunavir 1514 ng/ml (1061-1966) (n=40 samples, 25% quartile in Artemis study 368 ng/ml), maraviroc 53 ng/ml (46-58, efficacy threshold in Motivate 35 ng/mL) (n=63), ritonavir 74 ng/ml (58-106) (n=35). In 108 samples from 29 subjects regardless of collection time a significant positive correlation was found between maraviroc-darunavir (R=0.31; p=0.001), darunavir-ritonavir (R=0.57; p<0.0001), maraviroc-ritonavir (R=0.36; p <0.0001) levels. In univariate models age, gender, ethnicity, smoking, HCV+, BMI, NNRTIs, BPI, INI at screening or previously used, nadir and current CD4, FPR, adherence (>or<90%) were not associated with Ctrough of each drugs. Ctrough of darunavir and maraviroc below the median levels were not associated with an increased risk of a detectable HIVRNA.

Conclusions: In this switch study dosing MVC at 300 mg QD with DRV/r, median Ctroughs were in the range of efficacy in most cases and no correlation was found with detectable viral load during follow-up; the continuation of the study, currently still recruiting, is warranted.

No conflict of interest

Abstract: P_58

Novel Diagnostic Technologies & Approaches

Genotypic tropism testing on HIV-1 DNA in virologically suppressed patients and analysis of its determinants: results from the GUSTA study

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Background: Several studies have shown a good correlation between coreceptor tropism predicted through V3-loop DNA sequence of HIV-1 gp120 and the virological response to CCR5 antagonists. Correlates of DNA tropism results still require in depth exploration.

Materials & Methods: The GUSTA study randomizes patients on a successful antiretroviral therapy to switch to darunavir/r plus maraviroc or continuation of the original treatment. Enrolment criteria include HIV1-RNA <50 copies/ml for >6 months, R5 tropism and CD4>200 cells/ul for >3 months. Geno2pheno with 10% FPR cutoff was used to predict viral tropism on a single sequence of the HIV-1 DNA V3 region from whole blood. We evaluated the distribution and determinants of viral tropism, both as qualitative tropism classification and as quantitative FPR value, by univariable and multivariable logistic and linear regression, respectively.

Results: We included 116 patients: 83 were males (71.6%), 51 heterosexuals (44%), 101 Caucasian (87.1%), median age was 47,6 yrs (IQR 41-53), time from HIV diagnosis 11 yrs (7-17), CD4 630/ml (445-870), nadir CD4 156/ml (71-274), HIV-1 RNA was <50 copies/ml but detectable in 39 (33.6%), >50cp/ml in 5 (4.5%), negative in 68 (61%). Coreceptor tropism was predicted as R5 in 89 (76.7%), non-R5 in 26 (22.4 %), while 1 sample (0.9%) was not typeable. In a multivariable model adjusting for CD4 at nadir and time from HIV diagnosis, HIV-1 RNA detection (mean change in FPR+11.81, p=0.021) and current use of boosted PI (+12.79, p=0.032) were associated to higher FPR values. R5 tropism was detected in 85.7% of patients on boosted PI and 67.3% of patients not on boosted PI. Prior/current use of boosted PI was also the only variable independently associated with viral R5-tropism (OR 3.61; 95% CI 1.34-9.72, p=0.011) after adjusting for nadir CD4 and detectable HIV-1 RNA. Nadir CD4 levels showed a trend towards an association with R5 in the univariable analysis only (p=0.051).

Conclusions: The study has shown the feasibility of determining HIV-1 coreceptor tropism on viral DNA in virtually all patients with HIV-1 RNA< 50 cp/ml. The association between use of boosted PI and greater likelihood of R5 tropism advises exploring the combination of these drugs with CCR5 antagonists in clinical studies.

No conflict of interest

Abstract: P_59

Treatment Strategies for HIV/ Hepatitis infected Patients

High MBL serum levels is a predictive factor of failure to achieve successful response to treatment in Brazilian patients with HCV

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Background: Hepatitis C virus (HCV) is a widespread disease and the current treatment is only effective for a portion of the patients. Mannose-binding Lectin (MBL) is a protein that facilitates phagocytosis and lysis by activating complement when microorganisms invade the body. The purpose of this study was to examine which parameters could influence the response to treatment in patients with HCV. The focus was put on how the level of MBL could influence the response to treatment. It was hypothesized that too high levels of MBL can be harmful to the response to treatment due to a high level of inflammation caused by MBL.

Materials and Methods: Data from 551 Brazilian patients diagnosed with HCV was collected. The data collected was gender, age,

alcohol intake, presence of metabolic syndrome, stage of fibrosis, HCV genotype, amounts of different enzymes and the level of MBL. However only 123 of the patients had data about all these parameters and only these were used for the analysis. The data was collected before beginning the treatment and the treatment used was a conventional combination of interferon-α and ribavirin. All parameters were compared for a group of sustained virological responders (SVR) to a group of non-SVR. SVR means that these patients were HCV-RNA negative in their follow-up examination six months after the end of treatment.

Results: Univariable analyses were performed and significant associations were found between non-SVR and high stages of fibrosis (p=0.0282), high amount of gGT (p=0.001) and high amount of MBL (p=0.017). The relative risk to be non-SVR is 1.64 times higher with the increase of MBL levels.

Conclusions: A high level of MBL is significantly associated with failure to achieve successful response to treatment in Brazilian patients with HCV. This might be a result of a great amount of inflammation due to high activation of complement by MBL. It is hypothesized that the inflammation could interfere with the treatment.

No conflict of interest

Abstract: P_60

Treatment Strategies for HIV/ Hepatitis infected Patients

Prevalence of hepatitis C virus infection among HIV-infected people in Casablanca

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Introduction: HIV / HCV coinfection may have important implications for therapeutic and evolving plans. The Knowledge of its epidemiological, clinical features may help to anticipate needs and improve care. The aim of the Study was to determine the epidemic profile of patients co- infected by hepatitis C virus and HIV.

Materials and methods: A retrospective study was conducted in the infectious department diseases between 2003 and 201 3, were included all patients treated for HIV and HCV with positive serology(ELISA positive). Data were collected on computer files (Nadis) and analyzed on Epi Info.

Results: We collected 147 patients with HIV / HCV coinfection (4% of the total workforce). The average age was 40 ± 10 years, males predominated (4H/1F). Intravenous drug use was found in 29.25 % of patients. At the moment of diagnosis of the coinfection , 64 patients (43.5%) were in category C(CDC) . Antiretroviral therapy was prescribed for 131 patients (89%). The viral RNA research by RT-PCR and the genotyping were performed in 39 patients (27 %). The RNA HCV PCR was positive in 28 cases (19 %).The most frequent viral genotypes were 1a and 1b with 14 cases (50%). The IL28B performed in 8 patients had type CC (2 cases), C / T (3 cases) , TT (1 case) . Liver function tests were normal in 116 cases; liver cytolysis was found in 31 cases. the liver ultrasound was normal (80 cases) or showed lesions of chronic liver disease (30 cases) or ascites (10 cases) . Ten patients undergoing treatment with pegylated interferon and ribavirin. Two patients who completed treatment were non-responders. 79 patients (54%) are being used and 27 cases died (18%).

Conclusion: The HIV / HCV co-morbidity is a challenge in support of PLHIV. Currently, free access to treatment and explorations of hepatitis C in Morocco as part of a health program (RAMED) is an important step whose impact needs to be evaluated.

No conflict of interest

Abstract: P_61

Treatment Strategies for HIV/ Hepatitis infected Patients

Prevalence and characteristics of occult Hepatitis B infection in HIV-positive patients who did not receive HBV vaccine

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Background. Occult hepatitis B infection (OBI); presence of HBV DNA in the absence of HBsAg, has been described in a proportion of HIV patients with a wide range of variability. The exact mechanisms responsible for this event have been largely unknown. The aim of this study was to determine the general factors influencing cryptic HBV infection in a group of selected HIV patients.

Methods. A cross sectional survey was carried out on 211 HBsAg negative HIV-positive patients who did not receive HBV vaccine. Anti-HBc and anti-HBs were measured by enzyme-linked immunosorbent assay (ELISA). Real time PCR was applied to determine the levels of HBV DNA. They grouped into OBI positive (group I) and OBI negative (group II).

Results. 28 (13.2%, group-I) and 183 (86.8%, group-II) were OBI positive and negative, respectively. Despite some characteristics (age, gender, CD4 count, anti-HBs, anti-HBc, anti-HCV,....) showed variability between OBI positive *vs* OBI-negative groups, however, they did not reach significant relationship. 18, 12 and 90, 71 were anti-HBc positive and anti-HBs positive in groups I and II, respectively (P= 0.61 and 0.64, respectively); indication natural-resolved past HBV infection. 20 and 104 in groups I and II were anti-HCV positive, respectively (P= 0.53). Past history of risk factors (intravenous drug injection, transfusion, unprotected sex,..) showed no significant differences between both groups.

Conclusion. These results showed that the casual relationship between HIV OBI positive and negative patients might be influenced by some other (yet to be determined) factors. These factors may modify the interbalance between the virus and host immune system, leading to the cryptic nature of HBV in HIV patients.

No conflict of interest

Abstract: P_62

Treatment Strategies for HIV/ Hepatitis infected Patients

First case of recent retroviral infection after accidental occupational non-medical exposure to contaminated blood

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Background: In 2013, in our clinic has been applied post-exposure guidelines in 112 cases (health-care personnel of several hospitals in Bucharest) of occupational exposure to blood and other biological products and was not registered any cases of HIV transmission. We present the first case of recent retroviral infection after accidental occupational non-medical exposure to contaminated blood.

Case report: A 22 years old, young police-officer presented in our service for human bite wounds , resulting from an intervention. The aggressor, was known as drug-addicted, co-infected HCV/HIV and with bleeding in mouth, he was under antiretroviral therapy for about nine months (CBV+NVP , VL=90 787 copy/mL, CD4= 200 cell/mmc, RNA-VHC =6718917

UI/mL). Exposed -person comes to our service in the first 2 hours of the incident.
Guidelines for occupational exposure were applied , we excluded other risk factors for HIV infection and we started antiretroviral prophylaxis(CBV+LPV/r) given the regimen source and the high-risk of resistance mutations in terms of a poor adherence.
The initial evaluation to exposed-person was: HIV negative, HbsAg negative and anti-HBs positive.
At 8 days after initiation of post-exposure prophylaxis, exposed -person develops a generalized maculopapular rash and he goes to an emergency service; in the absence of other clinical manifestations the eruption is interpreted as post-drug side effects. The young men returns to our clinic after another 3 days (range in which in his own initiative stopped prophylaxis), and we decide changing antiretroviral regimen with DRV / r + TDF + FTC Revaluation after a month recorded that he remained asymptomatic and fourth generation ELISA tests were negative. Reassessment at 12 weeks shown serological tests positive (ELISA , Western blot -p24 = + +), indicating recent retroviral infection and we do the complete balance with CD4, HIV –RNA, specifying tropism and sub-type HIV-1, HCV -RNA and genotyping test.
We find the same HIV sub-type (F) and the same resistance mutations in those two patients, confirming HIV infection to policeman from the aggressor .New patient had initially HIV-RNA = 210 977 c / ml, CD4 = 789 cell/mmc and was included in the cohort of individuals with recent HIV infection of our institute, antiretroviral therapy is initiated with TDF + DRV / r + RAL. After a month of treatment HIV-RNA = 161c/ml blood, HIV- RNA from CFS was undetectable and anti-HCV were negative, normal transaminases.

Our case illustrates:

- The importance of adherence to antiretroviral medication;
- The consideration of acute retroviral syndrome in the presence of a maculopapular rash, even if it appeared under antiretroviral prophylaxis and in less than two weeks after the incident. The importance of choosing prophylactic regimen when we know treatment history and possible resistance mutations if the source-person is known to be HIV positive.
- Should be monitored more frequently and more complete (HIV-RNA), including people with occupational non-medical exposure .

No conflict of interest

Abstract: P_63

Treatment Strategies for HIV/ Hepatitis infected Patients

Relationship between concentrations of antiretroviral drugs in plasma and saliva

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Introduction: Therapeutic drug monitoring (TDM) is valuable to evaluate the efficacy of a regimen or monitor the compliance of patients treated with a drug. However, TDM requires frequent blood sampling, which carries a risk of viral transmission to medical staff and causes pain to patients. In contrast, saliva sampling is noninvasive, safe, cheap, and easily performed, even at home by the patient. We therefore examined the possibility of using saliva as an alternative body fluid for TDM.

Materials and Methods: We obtained blood and saliva samples from 30 human immunodeficiency virus (HIV)-1-infected outpatients who had been treated with darunavir (DRV), raltegravir (RAL), abacavir (ABC) or tenofovir (TDF) for >1 month and who provided informed consent to participate in this study. Eight patients were treated with a regimen containing DRV, nine with RAL, 16 with ABC and 13 with TDF. From each patient, blood and saliva samples were collected within 1 h of each other. Half of the plasma sample was centrifuged with an ultrafiltration device to obtain the non-protein-bound drug fraction (plasma ultrafiltrate). Drug concentrations in plasma, plasma ultrafiltrate, and saliva were

determined by liquid chromatography-tandem mass spectrometry.

Results: Drug concentrations in plasma and plasma ultrafiltrate showed plasma protein-binding ratios of 75.0% for DRV, 44.4% for RAL, 58.3% for ABC and <10.9% for TDF. Ratios of saliva concentration to plasma concentration were 6.5% for DRV, 13.5% for RAL, 62.3% for ABC and 2.4% for TDF. Significant correlations were evident between drug concentrations in saliva and those in plasma or plasma ultrafiltrate for DRV, RAL, and ABC. Correlation coefficients of drug concentration in saliva to plasma were 0.885 for DRV, 0.921 for RAL, 0.938 for ABC and 0.318 for TDF, and those in saliva to plasma ultrafiltrate were 0.922 for DRV, 0.947 for RAL, 0.976 for ABC and 0.332 for TDF. DRV, RAL, and ABC concentrations in saliva correlated more strongly with those in plasma ultrafiltrate than in plasma.

Conclusions: The possibility of using saliva for TDM was suggested for DRV, RAL, and ABC. In contrast, saliva cannot be used for this purpose with TDF, which exhibited a very low saliva concentration and no correlation between concentrations in saliva and plasma. Drug concentrations in saliva correlated better with those in plasma ultrafiltrate than with those in total plasma, suggesting that non-protein-bound drug may be secreted more easily to saliva. Among the drugs tested, ABC was most abundant in saliva. An ABC-containing regimen may be a candidate for pre-exposure prophylaxis against oral HIV transmission.

No conflict of interest

Abstract: P_64

Treatment Strategies for HIV/ Hepatitis infected Patients

Evolution of HIV-1 viral tropism and its relationship with CD4 and HIV-1 RNA: a prospective study in patients with acute infection and AIDS presenters

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Background: Acute infection and AIDS are more extreme phases of the natural history of HIV infection and the evolution of the virus. The acute infection represents a boost in plasma viremia, even before the immune system is able to prepare a neutralizing response to contain the infection. AIDS represents a decrease in immune competence after a stage of clinical latency. With this in mind, we analyzed the sequences from amplification of the V3 region of gp120 within the *env* gene and the correlation with the development of immuno-virological profile of patients in our study.

Materials & Methods: We enrolled 36 patients with acute/recent infection (n=20) or AIDS presenters (n=16). V3 sequences were obtained and co-receptor tropism was predicted using the Geno2pheno[coreceptor] algorithm. We analyzed various immuno-virological parameters in relation to the initial tropism of the virus: HIV-RNA, CD4+ cells, (count and percentage) at baseline, at 6 and 12 months; CD8+ cells (count) and CD4+/CD8+ ratio at baseline; HIV-RNA zenith and CD4+ cells nadir. Phylogenetic analysis was performed using bioinformatic tools.

Results: Our results demonstrated that a reconstitution of the immune system, evaluated as absolute recovery of CD4+ cells from baseline to six months in both patient groups, showed a more favorable trend in patients with R5 compared to X4. This was most evident in acute/recent infection (R5: 256.5 cells/μL; X4: 114 cells/μL) compared to the advanced stage of AIDS (R5: 81 cells/μL; X4: 71 cells/μL). Multivariate analysis performed to assess the independent determinants of immune reconstitution showed a correlation with the two variables of considerable interest in the group of acute/recent infections: a positive correlation between the level of CD4+ cells at baseline and the number of CD4+ cells during therapy at six months (p=0.001) and evidenced a trend in the association between R5 tropism and the number of CD4 cells in both arms, at 6 and 12 months after therapy initiation. Moreover, this analysis showed that, notwithstanding viral tropism, the gain in the number of CD4+ cells during therapy at six months had an inverse

correlation with the level of CD4+ cells at baseline (p=0.032). The risk associated with the advanced stage of the disease (*i.e.* CD4 counts ≤ 200 cells/µL in the arm of AIDS presenters), in accordance with the genotypic tropism, evidenced a trend in the association between low values of FPR and more advanced stages of the disease.

Conclusions: Evolution of the V3 hypervariable region of gp120 located within the *env* gene, determining the tropism of the virus, influenced the immuno-virological trend in the two cohorts of patients with an acute/recent infection and with an AIDS presentation. A recovery in CD4 cells was evident even with low CD4 cells at baseline with a prompt initiation of antiretroviral therapy. FPR values in the lower range showed a trend toward a serious impairment of the immune system.

No conflict of interest

Abstract: P_65

Treatment Strategies for HIV/ Hepatitis infected Patients

Monitoring HCV-treated patients using innovative software solution improves the general management of viral hepatitis C (HCV)

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Background: New direct active antiviral agents (DAAs) are increasingly being approved by European or American drug control institutions (like Sofosburir - sovaldi® - Gilead) for the treatment of chronic hepatitis C. Although such molecules proved their effectiveness, the related costs are still very high. Optimizing the follow-up of those patients and the delivery of these drugs using advanced software registries is a relevant topic to be addressed. We developed HepatiC, a centralized patient management registry used to optimally define patients' profiles combined with VisibleChek, a simple data querying and mining framework, currently being used by several networks of hepatologists worldwide.

Methods: Through collaboration with the Spanish Association of Liver Disease (AEEH) including eminent and recognized experts, we developed HepatiC, a secured web application tailored for an optimal management of patients suffering from chronic Hepatitis C. As registries need to be used by clinicians for a daily management of the patients but also be used at a global level (national, international) to perform epidemiological or surveillance studies, the software system was designed with a multi-sites approach where patients treated in several hospitals can be securely centralized in the same database. All key information including but not restricted to genotypes, co-factors, biopsies, fibrosis stage, pre-treatment, transplant information can be collected for both baseline and follow-up visits. All major DAAs can easily be initiated, discontinued, stopped using simple one-click actions and predefined parameters (like dosage, related adverse events...). The registry has been provided in 2013 to several centres in Spain to validate its usefulness, reliability and user-friendliness. For Epidemiology purposes, we developed VisibleChek, a data visualization and mining framework capable of querying any parameter collected through HepatiC.

Results: The first Spanish evaluation of HepatiC started in June 2013 and was originally restricted to a panel of 7 hospitals. More than 370 patients were recruited in a 3-months period and all relevant information were introduced in no more than 10 minutes per patient file. In October, HepatiC was publically announced by the AEEH and the access to the system was opened to any hospital in Spain. In January 2014, 500 patients (69% male, 31% female with a median age=56) coming from 12 different centres were captured including 370 treatment-experienced and 117 naïve patients. From this dataset 141 and 283 patients were treated with Boceprevir and Telaprevir respectively. From the patients screened for HCV genotypes, 350, 88, 47 and 2 patients

were identified as a 1b, 1a, 1, co-infection 1a+1b respectively. Moderate to Severe fibrosis stages were scrutinized using FibroScan, classifying 437 patients as F4 (230), F3 (132), F2 (41) and F0/F1 (34).

Conclusions: Providing to clinicians software applications tailored for the management of patients infected by HCV highly contributes to an optimized management of the disease at a patient level. In addition, combining those tools with advanced data mining applications help in global epidemiological and surveillance initiatives at national or international levels, for example to improve the cost-effectiveness of HCV-related treatments with patients stratification at baseline.

Conflict of interest Employee and shareholder of ABL

Abstract: P_66

Treatment Strategies for HIV/ Hepatitis infected Patients

Identification of a small HIV-1 neutralizing antibody fragment from the heavy chain CDR3 repertoire of an HIV-1 viremic controller

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Background: The recent identification of neutralizing antibodies able to prevent viral rebound reemphasized the interest in the humoral immune response in the control of HIV-1 infection. We identified a Viremic Controller presenting plasmatic neutralizing activity and characterized by more than 15 years of infection with a stable CD4 T cells count. In this study, the IgM- and IgG- derived heavy chain complementary determining region 3 (HCDR3) repertoires of this patient were displayed on filamentous phage to identify small antibody fragments inhibiting HIV-1 entry.

Methods: Plasma samples were analyzed for their ability to bind recombinant gp120 (LAI) in ELISA. Plasma neutralizing properties were assessed with viral infection assays using MT-4 cells with a CXCR4-using virus (III_B) and a single cycle infection assay based on luciferase-tagged recombinant viral particles (RVA) harboring HXB2 (-X4) envelope protein on U87-CXCR4 cells. Host genetic correlates of control were investigated by sequencing and HLA typing. Phage libraries displaying cyclic IgM and IgG derived HCDR3 peptides were engineered and screened against X4-tropic gp120 protein (LAI) immobilized on magnetic beads.

Results: Plasma samples of the Viremic Controller showed a strong IgG response against gp120 and inhibited up to 80 % of U87-CXCR4 cells infection at a 1/20 dilution in 4 longitudinal samples collected from 2006 to 2013. The patient genotyping revealed the absence of the CCR5 delta32 deletion or the CCR2 V64I- and SDF1 G801A- polymorphisms. HLA typing showed a combination of HLA A *02/*11, B*27/*35 and C*02/*04 alleles suggesting that protective CTL responses against HIV-1 might participate in viremic control. Screening of the IgM and IgG libraries allowed the isolation of 15 IgM HCDR3 sequences of which four were redundant and of a unique IgG sequence (LRTV-1) corresponding to the most frequently isolated IgM HCDR3 sequence. Interaction of LRTV-1 with gp120 was largely enhanced upon CD4 binding and sequence comparison between LRTV-1 and gp120-interacting partners (CD4, CXCR4/CCR5) revealed a high degree of similarity (11/23) between LRTV-1 and the C-terminus of the second extracellular loop of CXCR4 (ECL2-X4). Peptide LRTV-1 fully protected MT-4 cells (IC_{50}= 45 ± 1.6 µM) and U87-CXCR4 cells (-X4) (IC_{50}= 29 ± 5.1 µM). Alanine scanning showed that mutations of residues within the CDR3 sequence (P96, F100, W100A, I100D, F100E and D101 (Kabat numbering)) or framework sequences (C92A, C104A, F91A, A93F and W103A) decreased binding of LRTV-1 to gp120. Noteworthy, almost all these residues are conserved or share biophysical properties with the residues of ECL2-X4 critical for gp120 (LAI) binding.

Conclusions: From the IgM and IgG genetic repertoires of a Viremic Controller, we selected

a HCDR3 fragment binding to gp120 and inhibiting *in vitro* HIV-1 infection when expressed as peptide. Neutralizing antibodies targeting cryptic epitopes critical for co-receptor binding may be elicited as part of the control mechanisms in patients with low viremia. HCDR3 peptides offer a promising alternative to whole antibodies, scFv fragments and VHHs to target difficultly accessible protein sites with their small size, high affinity and selectivity and might become extremely relevant in the search of immunotherapeutic control of HIV infection.

No conflict of interest

Abstract: P_67

Treatment Strategies for HIV/ Hepatitis infected Patients

Clinical, virological and immunological features of newly HIV diagnosed patients in a medical reference area of Spain over the last 10 years: Still high, still late

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Background: To establish the best strategies for HIV diagnosis, prevention and treatment it is important to get a deep knowledge of the characteristics of newly HIV infections in our medical setting. Herein, we evaluated the clinical, virological and immunological status of newly HIV diagnosed patients in a medical care area of Spain over the last 10 years.

Methods: All newly diagnosed patients for HIV infection from 2004 to September 2013 at a reference medical care area attending 501,526 citizens were recorded. Epidemiological, virological, immunological and clinical data at the time of diagnosis were defined. The clinical outcome and response to antiretroviral therapy were also evaluated.

Results: A total of 531 newly HIV diagnosed patients were identified during the study period. The number of new cases was stable within the last 5 five years (62 cases/year). The majority were male (83%) and their mean age was 38 years. Routes of HIV transmission were: heterosexual 44%, homosexual (MSM) 38%, and intravenous drug users (IDU) 18%.
At the time of HIV diagnosis, median CD4 counts was 348 cells/µL and half of them (51%) had a median CD4 counts < 350 cells/µL. Overall, 33% of newly diagnosed HIV patients had an AIDS defining disease. Median HIV-RNA was 4.96 log cop/mL. HBV and HCV co-infection was recognized in 2.3% and 20% of newly HIV diagnosed patients, respectively.
Non-B subtypes were recognized in 34.2% of patients as follows: F (25.5%), C (2.9%), CRF02_AG (1.8%), A (1.8%), G (1.1%), CRF01_AE (0.7%), K (0.4%). Overall, the rate of transmitted drug resistance (TDR) over the study period was 6.4%, but a significant decreased to 3% was observed in the last 5 years (2009-2013). The most prevalent TDR mutations were: T215 revertants (1.8%), K219QENR (1.4%), for NRTIs; K103N (2.5%), for NNRTIs; L90M (0.4%), for PIs. After diagnosis, 75% of patients started antiretroviral therapy (ART) based on: 2NRTI+1NNRTI (47%); 2NRTI+1PI (45%); 2NRTI+integrase inhibitor (5%), and other regimens (3%). Median CD4 at ART initiation was 211 cells/µL. Overall, after one year of follow-up, 74.6% achieved undetectable viremia (HIV-RNA < 20 cop/mL). Multivariate analysis identified HIV-RNA < 100.000 cop/mL at diagnosis as the unique predictor of response to ART (OR 1.87 [1.28-2.58], p=0.002). During follow-up, 11% of patients died. The presence of an AIDS-defining disease at the time of diagnosis was significant associated with mortality (OR 2.97 [1.34-5.98], p=0.008).

Conclusions: The number of newly HIV diagnosed patients is stable since year 2008. There is a high prevalence of late diagnosis (51%) and 33% of them had an AIDS defining disease. The most prevalent non-B subtype was F (25.5%). Decreasing rates of TDR was observed from 10.4% (2004-2008) to 3% (2009-2013) in the last 5 years. Most of patients started antiretroviral therapy in the first year of diagnosis and the majority reached undetectable levels of viremia. These findings

support the need to facilitate the access for HIV testing to reduce the rate of late HIV diagnosis, improve the clinical outcome and prevent HIV transmission.

No conflict of interest

Abstract: P_68

Treatment Strategies for HIV/ Hepatitis infected Patients

Virological failure to a switch to Nevirapine, TDF, and FTC (or 3TC) in patients with suppression

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Background. The US DHHS antiretroviral guidelines warned against the combination of nevirapine (NVP) + tenofovir (TDF) + emtricitabine (FTC) (or lamivudine [3TC]) in naïve sowing to reports of early virological failure (VF) in several small studies. There are limited data regarding the long term efficacy and factors associated with VF of a simplification strategy consisting of a switch to NVP plus TDF/FTC (or 3TC) in HIV-1 infected patients with prior virological suppression.

Methods. In a systematic database search we identified all pre-treated patients who were switched to this combination in a tertiary hospital. No restriction criteria were included in the search. VF was defined as two consecutive plasma HIV-1 RNA measurements >50 c/ml. All baseline and follow-up genotypes were collected.

Results. We identified 341 patients: median age 42 years, 72% male, 37% MSM, 27% heterosexuals, 27% previous drug users, hepatitis C coinfection in 98, hepatitis B coinfection in 25, median baseline CD4 492 cells/mL, nadir CD4 229 cells/mL. The median time of HIV-1 infection was 6.1 years, median time on virological suppression (<50 c/mL) 48 months. Subjects had received a median of 6 prior antiretrovirals, and 79 had prior VFs documented. 146 (43%) had received prior mono or dual NRTI therapy. Patients stayed on the regimen for a median of 176 weeks. 96% had a VL<50 c/mL at 48 w and 86% in their last follow-up control. Confirmed VF was observed in 23 (6.7%) patients (17 3TC, 6 FTC, p=0.034). Population genotypes were available in all 23, with 5 showing wild-type HIV-1. 16/23 patients had NNRTI mutations (Y181C/I/D:10; K103N:6; V106A/I:3; Y188C/L: 2; K101Q/E: 2; M230L: 1; P225H: 1; A98G: 1; V108I: 1; F227L: 1; K238T: 1), 10 of them had >1 mutation. M184V was selected in 16 subjects (none with M184I);K65R in 7 patients (6 associated to M184V, none with thymidine-analogue mutations [TAMs]). Six patients selected A62V, none of them with Q151M. Five patients harboured TAMs. Factors associated with VF in multivariant analysis were prior NNRTI interruptions (HR 5,76; 1.11, 29.87), and number of prior NRTIs (1,49; 1.15, 1.93). Prior drug users were only associated with treatment failure (HR 1,15; 1.12, 2.04).

Conclusions. Rates of VF of a regimen composed of NVP plus TDF and FTC (or 3TC) started as a switching strategy in subjects with undetectable plasma HIV-1 RNA are low, similar to other simplification strategies, and linked to some known baseline variables. Among failures, M184V and NNRTI mutations are highly prevalent, and rates of K65R selection low, but higher than those reported for efavirenz (when associated to TDF and FTC/3TC). Resistance selection rates are higher with 3TC than FTC. These data do not support a caution warning against this regimen in simplification.

No conflict of interest

Abstract: P_69

Treatment Strategies for HIV/ Hepatitis infected Patients

Nucleos(t)ide analog sparing regimen:efficacy of atazanavir/r plus maraviroc in HIV/HCV coinfected patients with suppressed plasma HIV1-RNA viral load

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Background: Switching to nucleos(t)ides (Nuc) sparing regimen in HIV patients represents a valid option to avoid drug toxicities. Few data are achievable about atazanavir/ritonavir (ATV/r) plus maraviroc (MVC) association particularly in HIV/HCV co-infected patients with non detectable HIV-RNA. ATV/r has good efficacy and tolerability in treatment-naive and experienced patients with a low impact on lipids and glucose metabolism. MVC is effective, safe and seems to slow the liver fibrosis progression in HIV/HCV co-infected subjects. Aim of this study was to evaluate patients on suppressive antiviral therapy (ART) switching to an ATZ/r plus MVC regimen and test the hypothesis that the switch may have an impact on liver fibrosis progression.

Material & Method: In this pilot study, 10 treated adults with an undetectable HIV-1-RNA plasma viral load (VL < 50 copies/ml) and with a detectable HCV-RNA who switch to MVC 150 mg BID plus ATV/r 300/100 mg QD, followed in a single centre of Infectious Diseases in Brescia, Italy, have been retrospectively analysed. Viral tropism was assessed by Geno2Pheno software. Changes in clinical and laboratory parameters were evaluated at a median of 6, 12 and 18 months of follow-up. Liver fibrosis progression has been measured with FIB4 score [(Age x AST) / (Platelts x (sqr (ALT)].

Result: 4 female and 6 male patients were observed for median 9 months. Reasons for switching the ATV/r plus MCV regimen were: renal impairment (GFR< 80 ml/min) (n= 3/10), bone mineral density reduction (defined by DEXA scan) (n= 4/10), ART simplification (n=2/10), and virological failure (n=1/10). After the switch CD4+ T cell count increased from 532 cell/mm^3 (IQR 237-664) to 709 cell/mm^3 (112-723). Nine patients maintained a VL below 50 copies/ml; one patient who failed the previous regimen did not experience the VL suppression because of poor compliance also with the new regimen. During the observation (from baseline to the end of follow up) the median values of following laboratory parameters have been reduced: ALT [from 106 UI/l (IQR 43-142) to 55 UI/l (21-89)]; AST [from 62 UI/l (44-130) to 48 UI/l (24-72)]; GGT [from 85 UI/l (45-140) to 65 UI/l (36-95)]; total cholesterol (from 159 mg/dl (129-170) to 114 mg/dl (66-162)]; Triglycerides [from 133 mg/dl (94-141) to 66 mg/dl (65-68)]; glycaemia [95 mg/dl (81-101) to 92 mg/dl (86-98)]. A FIB4 score reduction has been observed [2.7 (0,9-9,2) to 2.4 (0,7-16,2)]. The bilirubin value did not increase: 1.9 mg/dl (IQR 0.9-2.9) at the baseline e 1.5 mg/dl (1.2-1.8) at the end of follow up.

Conclusions: This is the first report about the MVC plus ATV/r association in HIV/HCV co-infected patients with undetectable HIV-viral load. It is could be a reasonable new option to avoid long term NUC toxicity particularly in fragile patients. Further investigations and longer time of follow up will help to clarify the advantages of this combination.

No conflict of interest

Abstract: P_70

Treatment Strategies for HIV/ Hepatitis infected Patients

High prevalence of subtype F in newly diagnosed HIV-1 persons in northwest Spain and evidence for impaired treatment response

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Background: The prevalence of non-B subtypes is increasing in Western countries largely as result of population movements. The most common non-B variants in Spain are CRF02_AG and subtype G. Due to strong links overseas, the HIV epidemic in Galicia has been historically characterized by a broader spectrum of HIV variants.

Methods: All newly diagnosed HIV-1 persons from 2004 to 2013 at our institution, which covers 501,526 citizens in Northwest Spain were identified. All data at the time of diagnosis as well as response to ART were analyzed.

Results: A total of 531 newly diagnosed HIV-1 patients were identified during the study period. HIV subtypes were determined in 275. Non-B variants were found in 34.2%, distributed as follows: F (25.5%), C (2.9%), CRF02_AG (1.8%), A (1.8%), G (1.1%), CRF01_AE (0.7%) and K (0.4%).
A comparison was performed between subtypes B (n=182) and F (n=70). Subtype F predominated in men who have sex with men (MSM) compared to subtype B (79% vs. 41%, p<0.001). At diagnosis, no differences were found in mean age (36.7 vs. 39.5 years), AIDS defining disease stage (23.5% vs. 30.2%) and proportion with CD4 <350 cells/µL (41.4% vs. 46.2%). Likewise, median HIV-RNA did not differ significantly between F and B (5.3 vs. 4.9 log cop/mL), despite being lower mean CD4 counts (375 vs. 417 cells/µl, p=0.01).
After diagnosis, 86.4% of subtype F and 76.4% of subtype B patients initiated ART based on: 2NRTI+1NNRTI (56.1% vs. 46.3%); 2NRTI+1PI (33.3% vs. 47.8%); 2NRTI+1INI (8.8% vs. 6%). Interestingly, a lower proportion of subtype F vs B reached plasma HIV-RNA <50 cop/mL at weeks 24 (34.9% vs. 76.6%), 48 (51.7% vs. 85.2%) and 96 (61.1% vs. 100%). In multivariate analysis, subtype F (OR 9.2 [2.44-36.61], p<0.001), baseline HIV-RNA (OR 3.7 [1.23-10.93], p=0.019), and ART initiation delay after diagnosis (OR 1.1 [1.02-1.10], p=0.004) were independent predictors of virologic response to ART at 24 weeks.

Conclusions: Subtype F is the most prevalent non-B subtype among newly diagnosed HIV-1 persons in Northwest Spain, with preferential transmission among MSM. The lower virological response to ART in subtype F vs B warrants further investigation.

No conflict of interest

Abstract: P_71

Treatment Strategies for HIV/ Hepatitis infected Patients

Negligible clinical relevance of CYP2D6 and CYP3A4 polymorphisms for metabolic turnover rate and therapy success of HIV protease inhibitors

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Background: Inadequate plasma levels of antiviral drugs despite therapy-compliance represents a major issue observed in clinical routine. There is little knowledge about the impact of polymorphisms in drug transporter proteins and hepatic drug metabolizing enzymes like cytochrom P450 superfamily members CYP2D6 and CYP3A4 on metabolic turnover rates of antiviral drugs like HIV protease inhibitors (PI) in vivo.

Methods: 64 therapy-experienced patients were screened for CYP2D6*XN high expression alleles and CYP3A4*18 alleles. Data of HIV viral load and plasma drug levels (Cmax and Ctrough) of HIV PIs were documented and compared according to their allele status. Cmax plasma concentrations were recorded 4 hours and Ctrough levels 12 hours after intake. Of all 64 patients with RTV-boosted therapies, 18 patients received DRV-, 21 patients LPV-, 15 patients ATV- and 12 SQV-containing therapies (including two patients with double PI).

Results: CYP3A4 polymorphisms, that could affect drug metabolism negatively, were not detected in any of the 64 analyzed samples.

Alterations in CYP2D6 were found in six out of 64 patients [9.4%]. Ritonavir plasma levels, that are mainly affected by CYP2D6 activity, ranged from mean trough levels of 154 to max. levels of 494 ng/ml and did not differ significantly from patients without alterations in CYP2D6 concerning plasma levels and therapy success. LPV levels ranged from trough levels of 2,840 ng/ml to max. levels of 6620 ng/ml in these patient group; for DRV from 2,750 ng/ml to 7,610 ng/ml, respectively and for ATV from 925 ng/ml to 5050 mg/ml, respectively. SQV was not prescribed in this patient group.

Conclusions: High expression alleles in cytochrome P450 family members CYP2D6 and CYP3A4 occur rarely in clinical routine. None of all 64 analyzed patients were tested positive for CYP3A4*18 polymorphism, which indicates a minor role of this event on protease inhibitor plasma levels. The CYP2D6*XN high expression allele can be observed in approximately 10% of patients, however there was no indication for a clinically relevant impact of this alleles on ritonavir plasma levels or other protease inhibitors in this study.

No conflict of interest

Abstract: P_72

Treatment Strategies for HIV/ Hepatitis infected Patients

Cellular localization and dimerization properties of the N-terminal transmembrane domain of the HCV NS2 protease

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Background: Direct-acting antivirals (DAAs) have shown high potency and a good tolerability profile for HCV infection. In this context, the Non-Structural protein 2 (NS2), a membrane-associated cysteine protease, is involved in the first step of the viral polyprotein processing and represents thus a promising target for the development of new DAAs. In the polyprotein, the NS2 N-terminal part ($NS2^{N\text{-}term}$) is preceded by the p7 protein and embedded in the endoplasmic reticulum membrane, while its C-terminal part ($NS2^{pro}$), the protease domain, is located in the cytoplasm. The $NS2^{pro}$ domain adopts a unique dimeric structure characterized by two composite active sites, which are formed by catalytic residues belonging to both monomers and dimerization of which is a prerequisite for the proteolytic activity and the further release of the NS3 protease. In this study, the exact role of $NS2^{N\text{-}term}$ in the cellular localization and the initiation of the NS2 dimerization process has been elucidated.

Material and methods: Sequences encoding the N-terminal domains of NS2 (1-90, 1-95 and 1-100) were engineered based on the genotype 1a strain H77 sequence and cloned into pCMV vector upstream of the FRET donor (Cerulean) or FRET acceptor (Venus) sequences. Vectors lacking or presenting the complete NS2 sequence ($NS2^{Nterm(1\text{-}100)}$+$NS2^{pro}$) were constructed as negative and positive control, respectively. In all constructs a sequence encoding a flexible linker (GGGSGGGS) was inserted to separate the NS2 segments from the fluorescent proteins. Huh-7D cells were transfected with individual or pairs of pCMV vectors and the expression and localization of each individual fusion protein was evaluated using confocal fluorescence microscopy. FRET signal was recorded using a Zeiss LSM510 META microscope 24h and 48h post transfection by measuring the enhancement of the Venus fluorescence intensity.

Results: All $NS2^{Nterm}$ fusion proteins were expressed at a similar level in Huh7D cells, regardless their length. Cellular localization revealed that all NS2 constructs were correctly addressed and maintained in the endoplasmic reticulum even in the absence of the p7 protein. In contrast, the negative controls (Cerulean or Venus alone) were detected in the whole cytoplasm. Interestingly, FRET intensity values measuring the efficacy of protein dimerization obtained with the three $NS2^{Nterm}$ constructs were similar to that observed with the complete NS2 ($NS2^{Nterm+pro}$). The specificity of the

detected interactions was confirmed by assessing the independence of FRET of the fluorescence intensity.

Conclusions: The N-terminal transmembrane domain of the NS2 cysteine protease is sufficient to address and retain the protein in the endoplasmic reticulum of the transfected cells. $NS2^{N\text{-}term}$ domain is able to dimerize, even in the absence of any other viral protein, suggesting that this domain could also play an important role in the initiation of the $NS2^{pro}$ dimerization. Our results provide new insights on the molecular and functional roles of the NS2 N-terminal domain and allow a better understanding of the initial steps of the HCV polyprotein processing. The $NS2^{Nterm}$ domain could therefore represent a new valuable target for DAAs.

No conflict of interest

Abstract: P_73

Treatment Strategies for HIV/ Hepatitis infected Patients

Safety of HIV-1 therapy during HCV treatment with different DAA in a Berlin´s cohort of HIV/HCV co-infected patients

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Background: Because of drug-drug interactions of HIV- and HCV-specific treatments initiating a HCV-therapy the antiretroviral therapy (ART) often has to be changed. The spectrum of applicable HIV-drugs is small, therefore we investigated the ART outcome in co-infected patients starting an HCV treatment with DAA (direct acting agents).

Materials & Methods: Overall 29 patients initiating DAA containing HCV therapy (12x Faldaprevir, 9x Telaprevir, 4x Daclatasvir and 4x Simeprevir) were monitored retrospectively concerning HI-viral load and CD4+ cell count before, during and after HCV treatment.

Results: 19 patients were switched to TDF/FTC+RAL, three and two retained their ATV/r or DRV/r based regimen, respectively. One switched to a new ATV/r, DRV/r or RAL containing regimen, respectively. Two patients did not receive an ART. Success of HCV therapy was shown by 16 patients with sustained virological response (SVR), 10 without SVR (some due to re-infection within one year) and three patients were lost to follow up. No HIV-1 viral breakthrough was observed, but one complete therapy interruption. The two patients without ART showed an initial decrease in VL followed by a re-increase after interferon withdrawal. Mean CD4+ cell counts rose from 28% before HCV treatment, to 32% after 12 weeks and 36% after 24 weeks, and 32% to the end of HCV treatment, but dropped in absolute numbers from 558 cells/µl before, to 354 and 386 cells/µl after 12 and 24 weeks, to 416 cells/µl to end of HCV treatment, respectively. The effects revised after end of HCV therapy to 30% and 623 CD4+ cells/µl.

Conclusion: Changes in HIV ART due to initiating HCV therapy was safe for the observed cohort even with the limited drug choice of antiretrovirals not interacting with DAA containing regimen. The interferon´s anti-viral effect suppressed HIV VL even without ART below detection limit to some extent

Conflict of interest :Financial relationship(s): ViiV health care, Diasorin, AbbVie, MSD, GILEAD sciences

Abstract: P_74

Treatment Strategies for HIV/ Hepatitis infected Patients

Different kinetics of HIV replication and DNA integration in vitro within the two major cellular reservoirs in presence/absence of integrase inhibitors

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Introduction: The goal of the study was to compare the kinetics of HIV-DNA integration, p24 production and the equilibrium between provirus and unintegrated-forms (UF) of viral-DNA in primary macrophages and lymphocytes, in presence and absence of integrase inhibitors (INIs).

Methods: Monocyte-derived-macrophages (MDM) and CD4+ Tcells were obtained from human primary peripheral-blood-mononuclear-cells (PBMC) and were infected with HIV-1 (p81.A for MDM and PBMC, pNL4.3 for lymphocytes), in presence or absence of INIs (Raltegravir and Dolutegravir). Viral replication was assessed by ELISA analysis of the p24 production. Quantification of HIV-provirus, UF and 2-LTR-circles was performed by real-time PCR after chromatographic separation of HIV-DNA UF from the proviral high-molecular-weight-DNA, at different time post-infection (for MDM: 1h, 4h, 24h, 48h, 72h, 5d, 7d, 14d, 21d, 30d post-infection and PBMC and CD4+Tcells: 1h, 4h, 24h, 72h, 5d, 7d post-infection).

Results: In CD4+T-cells and PBMC, in absence of INIs, all HIV-DNA forms were very low and stable up to 72h with very little p24-production (CD4+T-cells total-DNA ≤12cps/1000cells and p24 ≤1.5ng/ml; PBMC total-DNA ≤9cps/1000cells, p24 ≤0.2ng/ml), then an exponential increase was observed over time, reaching the highest levels at 7dpi (CD4+T-cells proviral-DNA =583cps/1000cells and UF-DNA =338cps/1000cells; PBMC proviral-DNA =440cps/1000cells and UF-DNA =350cps/1000cells), with a corresponding p24 production of 218ng/ml and 159ng/ml, respectively. Of interest, the provirus/UF ratio changed over time (from 16:1 at 1hpi to 2:1 at 7dpi) showing an increase of the UF levels. In presence of INIs (100nM), starting from 48h post-infection and at all time-points, total HIV-DNA was always ≤2cps/1000cells in both CD4+T-cells and PBMC (median 2-LTR/UF = 48% and 10%, respectively).
In macrophages, in absence of INI, the HIV-DNA slope was differently shaped. All HIV-DNA forms were very low and stable up to 48h (≤40cps/1000cells), then an exponential increase was observed over time. At 72h, total HIV-DNA increased (54.3cps/1000cells), reaching the highest levels at 21dpi (proviral-DNA =1580cps/1000cells and UF-DNA =616cps/1000cells). A similar curve was observed with p24 (220-231ng/ml at 14-30dpi). Also in MDM, the provirus/UF ratio changed overtime (from very low until 72hpi ≤0.1, to 1.5:1 at 30dpi, with a spike of 5:1 at 14dpi) showing an increase of the UF levels at 30days. Starting from 48hpi, in resting MDM, INIs determined a strong HIV-DNA reduction (mean percentage <79%) compared to the mock-treated cells (100%). However, a minor still consistent detection of HIV-DNA (both provirus and UF) was always found in treated MDM, starting from 14dpi up to 30dpi (median total HIV-DNA =3.9cps/1000cells, median 2-LTR/UF=87%).

Conclusions: Both lymphocytes and macrophages are productively infected and stably contain provirus and a minor rate of UF viral-DNA. However, a fast infection turnover was observed in T-cells compared to the persistence of chronic infection in MDM. Both INIs are potent inhibitors of HIV integration and replication in both the two major HIV cellular reservoirs. Finally, the limited HIV-DNA stably detected in treated-MDM deserves further investigation to define its pathogenic and clinical significance.

No conflict of interest

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